Journal of the Fisheries Research Board of Canada

Journal de l'office des recherches sur les pêcheries du Canada

Volume 36, No. 5, May 1979

Volume 36, nº 5, mai 1979

Fisheries and Oceans Canada

Pêches et Océans Canada

Journal of the Fisheries Research Board of Canada

Journal de l'office des recherches sur les pêcheries du Canada

Fisheries and Oceans Canada
Scientific Information and Publications Branch
Ottawa K1A 0E6

Pêches et Océans Canada
Direction de l'information et des publications scientifiques
Ottawa K1A 0E6

Volume 36, No. 5, May 1979

Volume 36, nº 5, mai 1979

The *Journal of the Fisheries Research Board of Canada* has been published continuously since 1901, previously as *Contributions to Canadian Biology* 1901–25, *Contributions to Canadian Biology and Fisheries* 1926–34, and *Journal of the Biological Board of Canada*, 1934–37.

Editorial policy — The *Journal* publishes original research articles and notes, critical reviews, essays of opinion or hypothesis, letters, and book reviews in the broad field of fisheries management and technology, ocean science, and aquatic environments relevant to Canada. Submissions in English or French are accepted without limitations as to origin, but must not have been published or copyrighted and must not be published elsewhere except in abstract form or by written consent of the Editor. The *Journal* accepts no responsibility for statements made by contributors. The use of proprietary names does not imply endorsement of the product or company. Guides for authors and typists appear in the first issue of each volume and are available free from the *Journal*.

A charge of $44 per page is made for manuscripts published. When submitting a manuscript, an author must state that the charge will be accepted.

Subscriptions — The *Journal* is issued monthly to form annual volumes of twelve numbers. Subscription rates are: Canada, $40 per year; other countries, $48. Single issues are $4.00 and $4.80, respectively. Orders must be sent directly to Supply and Services Canada, Ottawa, Canada, K1A 0S9, and payment made in Canadian funds to "The Receiver General for Canada."

Microfilm — Issues from 1934 through the current volume can be purchased on 16 or 35 mm microfilm. Photocopies of individual articles or issues can be purchased. Address orders to Xerox University Microfilms, 300 North Zeeb Road, Ann Arbor, Michigan 48106, USA.

Publié depuis 1901, le *Journal de l'office des recherches sur les pêcheries du Canada* a paru sous plusieurs titres: *Contributions to Canadian Biology*, 1901–25, *Contributions to Canadian Biology and Fisheries*, 1926–34, et *Journal of the Biological Board of Canada*, 1934–37.

Politique de publication — Le *Journal* publie des articles de recherches, ainsi que des notes, critiques, exposés d'opinions ou d'hypothèses, lettres et recensions concernant le vaste domaine de la gestion des pêches et de la technologie, des sciences de la mer et des milieux aquatiques au Canada. Toutes contributions en français ou en anglais sont acceptées sans restriction quant à la provenance, mais elles ne doivent pas avoir été produites ni avoir fait l'objet d'un copyright et ne doivent pas être publiées nulle part ailleurs sauf sous forme abrégée ou avec le consentement écrit du directeur. Le *Journal* décline toute responsabilité pour ce qui est des affirmations des collaborateurs. L'utilisation de noms de marque de commerce ne signifie aucunement une sanction du produit en question ou de la compagnie qui le fabrique. Le guides des auteurs et le guide des dactylos paraissent dans le premier numéro de chaque volume et sont disponibles gratuitement du *Journal*.

Les frais de publication sont de $44 la page. Quant un auteur présente un texte, il doit préciser que ces frais seront acceptés.

Abonnement — Le *Journal* paraît tous les mois, formant ainsi un volume de 12 numéros par année. Les frais d'abonnement annuel sont de $40 pour le Canada et de $48 pour les autres pays. Chaque numéro coûte $4.00 au Canada et $4.80 à l'étranger. Prière d'adresser directement les commandes à Approvisionnements et Services Canada, Ottawa (Ontario), Canada K1A 0S9; les paiements doivent être faits en devises canadiennes, à l'ordre du Receveur général du Canada.

Microfilms — On peut acheter sur microfilms 16 ou 35 mm tous les numéros publiés depuis 1934. Des photocopies d'articles ou de numéros individuels sont aussi en vente. Prière d'adresser les commandes à Xerox University Microfilms, 300 North Zeeb Road, Ann Arbor, Michigan 48106, E.-U.

Editor and Director of Scientific Information/ Rédacteur et Directeur de l'information scientifique — J. C. STEVENSON, PH.D.

Deputy Editor/ Sous-rédacteur — J. WATSON, PH.D.

Assistant Editors/ Rédacteurs adjoints — D. G. COOK, PH.D./ LORRAINE C. SMITH, PH.D.

Production-Documentation — J. CAMP/ G. NEVILLE/ PAULA IRVING/ A. T. REID

French Abstracts/ Résumés français — Y. JEAN, PH.D.

Book Review Editor/ Rédacteur des comptes rendus — D. P. SCOTT, PH.D.

Journal of the Fisheries Research Board of Canada

Journal de l'office des recherches sur les pêcheries du Canada

Volume 36, No. 5, May 1979

Volume 36, nº 5, mai 1979

Timing and Magnitude of Energy Deposition and Loss in the Body, Liver, and Gonads of Northern Pike (*Esox lucius*)

JAMES S. DIANA AND W. C. MACKAY

Department of Zoology, Biological Sciences Centre, University of Alberta, Edmonton, Alta. T6G 2E9

DIANA, J. S., AND W. C. MACKAY. 1979. Timing and magnitude of energy deposition and loss in the body, liver, and gonads of northern pike (*Esox lucius*). J. Fish. Res. Board Can. 36: 481–487.

Northern pike (*Esox lucius*) were sampled periodically from Lac Ste. Anne, Alberta, between March 1976 and September 1978. The yearly cycle of production and depletion was determined for individual 3-yr-old fish. Body growth in length was similar for both sexes, and occurred during the summer. Somatic caloric growth was completed during the summer by females, while male somatic growth continued over winter. Gonad growth began in August; testicular growth was completed by September while ovarian growth occurred mainly during winter. There was no loss of somatic energy during ovary growth. Energy requirements for testicular growth appeared to come from liver stores. Both sexes lost considerable somatic energy between March and May, the spawning period; this loss appeared to be due to spawning activity and not late gonad growth. Young-of-year fish did not mature sexually and all of their energy accumulation went into somatic tissue. Total production in females from May to March was twice as high as production in males. Both sexes had similar yearly somatic production, while females had approximately 15 fold higher gonadal production than males.

Key words: calories, growth, liver, gonad, somatic, *Esox lucius*, production, spawning depletion

DIANA, J. S., AND W. C. MACKAY. 1979. Timing and magnitude of energy deposition and loss in the body, liver, and gonads of northern pike (*Esox lucius*). J. Fish. Res. Board Can. 36: 481–487.

Des échantillons de grands brochets (*Esox lucius*) ont été capturés périodiquement dans le lac Ste-Anne en Alberta entre mars 1976 et septembre 1978. Chez des sujets de 3 ans, nous avons déterminé le cycle annuel de production et de déplétion. La croissance corporelle en longueur est semblable chez les deux sexes et a lieu en été. La croissance calorique somatique se termine en été chez les femelles, alors que cette croissance se poursuit en hiver chez les mâles. La croissance gonadique commence en août; la croissance testiculaire est terminée en septembre, alors que la croissance ovarienne a lieu principalement en hiver. Il n'y a pas perte d'énergie somatique durant la croissance ovarienne. Les besoins énergétiques de la croissance testiculaire semblent provenir de réserves du foie. Chez les deux sexes, il y a perte considérable d'énergie somatique entre mars et mai, la période de fraie; cette perte semble résulter de

Printed in Canada (J5251)
Imprimé au Canada (J5251)

l'activité reproductrice et non d'une croissance tardive des gonades. Les jeunes de l'année ne mûrissent pas sexuellement et toute leur accumulation énergétique passe dans le tissu somatique. La production totale chez les femelles, de mai à mars, est le double de celle des mâles. Les deux sexes ont une production somatique annuelle semblable, alors que les femelles ont une production gonadique 15 fois supérieure à celle des mâles.

Received May 19, 1978
Accepted January 17, 1979

Reçu le 19 mai 1978
Accepté le 17 janvier 1979

THE most important processes in the life of any animal are the accumulation of energy and its use in body growth, reproduction, and activity. To estimate total energy production for individual fish, the absolute quantity of energy in the entire body of many fish must be determined during several different periods of the year. The energy dynamics of fish were first examined by Idler and Bitners (1958, 1960) for migratory sockeye salmon (*Oncorhynchus nerka*). They converted data from fish of all sizes to a standard 50-cm fish, which was reasonable in their analysis since there was no evidence that salmon grew during the freshwater phase of spawning migration. However, this "standard fish" technique has been used by some authors (MacKinnon 1972; Craig 1977; Medford and Mackay 1978) to analyze year-round changes in individual fish. This type of analysis underestimates production, since the fish grow both in length and weight. Studies by Healey (1972) and Foltz and Norden (1977) have examined energy production in fish, taking growth in length into account.

The timing of various functions, such as body growth, gonad growth, and spawning is very precise from year to year (Love 1970; Shul'man 1974). There may be large differences between the sexes in the amount of energy required for gonad growth and maturation (Love 1970; Shul'man 1974), because ovarian tissue usually contains much more energy than testicular tissue. Northern pike (*Esox lucius*) conform to the above pattern, and ovarian growth continues during winter, while testicular growth is completed in late summer (Huang and Hickman 1968; Medford and Mackay 1978). These differences in the magnitude and timing of gonad growth between sexes of pike must have considerable influence on the pattern of energy use. In addition, the proportional allocation of energy to body and gonad growth may vary with age.

The purpose of this study was to determine the timing and magnitude of energy deposition and depletion in the body, liver, and gonads of northern pike during the year. A sufficient number of fish were sampled to allow growth in length and in total calories to be determined for several age-classes of pike. The effect of age, season, and sex on growth was examined to allow a better understanding of the production process in individual fish.

Materials and Methods

Lac Ste. Anne (114°24′W, 53°43′N) is a large eutrophic lake ~74 km northwest of Edmonton, Alberta. The lake is 57 km² in area, with a maximum depth of 10 m and an average depth of 4.8 m. The water is usually isothermal, with temperatures fairly constant during the summer (14–16°C from May to October) and winter (1°C from November to April). Only during brief periods in October and April do water temperatures differ much from one of these two regimes.

Northern pike were collected by gillnet from Lac Ste. Anne on 26 occasions between March 1976 and September 1978, except for April 1977 when they were collected by seine from a tributary stream. The fish were frozen at −25°C and thawed later for analysis. The gross body weight was measured to 0.1 g, and the standard length to 0.5 cm. Four pike from each sex and age-class (when possible) were selected from samples collected on six occasions between March 1976 and May 1977 and used for caloric analysis. The body (fish without liver, gonads, or digestive tract), liver, and gonads of each fish were dried to constant weight at 80°C, ground to powder in a coffee mill, then stored in vials at −25°C until later analysis. Body samples were passed through a meat grinder before drying.

Calorific equivalents (kcal/g dry wt) were determined using a Parr adiabatic calorimeter following standard methods (Paine 1971). A mean (±2 SD) value of 5.0 ± 0.02 kcal (1 kcal = 4186 J) per gram was obtained from six replicates of one somatic sample. Because of this precision, only one determination was done per sample. Corrections were made for burnt fuse and nitric acid during each run, but sulphur corrections were omitted.

The ash content of somatic tissue was determined by heating ~1 g of sample to 500°C for at least 1 h in a muffle furnace (Paine 1971). The sample was then allowed to cool and weighed.

Pike were aged by counting the number of annuli on scales taken from the left side of each fish, dorsal to the lateral line and just anterior to the dorsal fin (Frost and Kipling 1959). Approximately 5% of the fish could not be aged by scales and were classed with similar sized fish from the same sample that had been aged.

The total energy content of pike tissues are given for 3-yr-old fish in this text rather than attempting to fully describe yearly growth in each age-class. Three year olds were used because they were well represented in the samples and they all had undergone one spawning cycle, so their gonadal maturation process was more distinct than that of younger fish.

Seasonal and age-related changes in energetic parameters were determined by analysis of variance and the Student–Newman–Keuls test. Differences were considered significant if $P < 0.05$. Analysis was done on the University of Alberta computer using programs from the SPSS package (Nie et al. 1975).

Results

Body Growth

Significant growth in length by adult northern pike

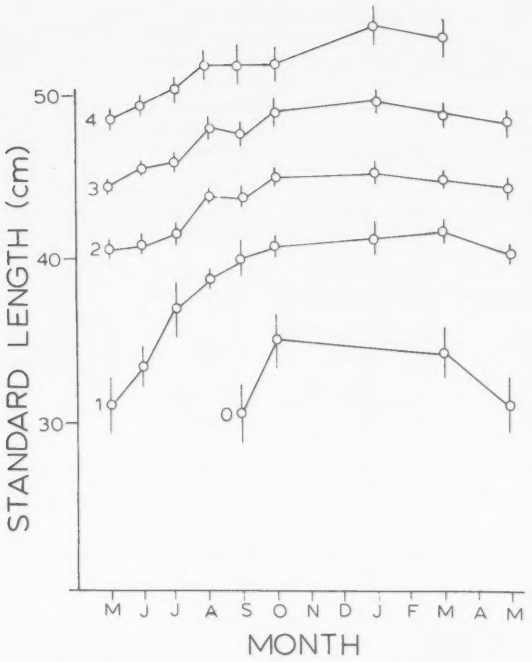


FIG. 1. Mean standard length of each age-class of northern pike ($\bar{x} \pm 2$ SE) collected monthly in Lac Ste. Anne from 1976 to 1978. Age is indicated by the number before each group. Values are based on 1365 pike with a mean of 34 fish/group (range 3–72).

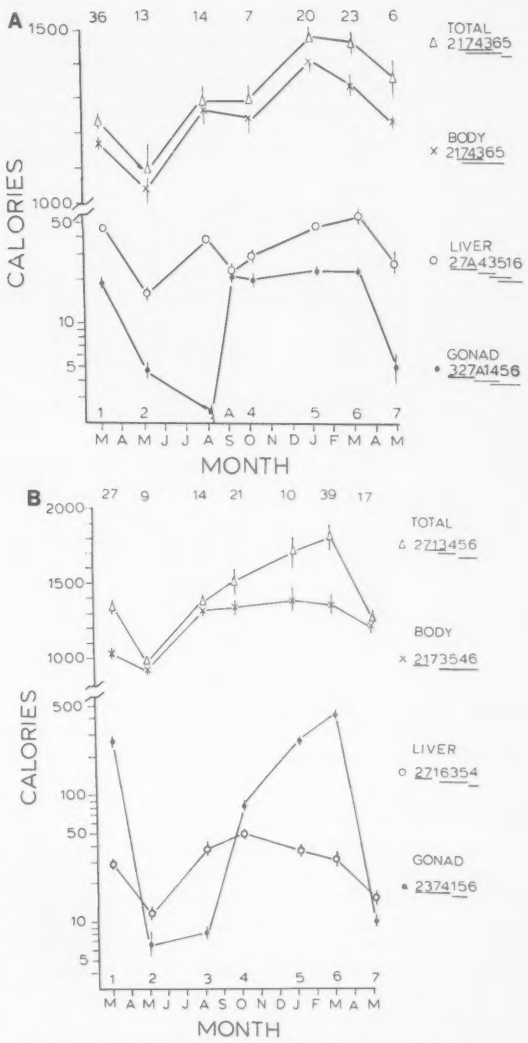


FIG. 2. Caloric (1 kcal = 4186 J) content of the liver (open circles), gonad (closed circles), body (crosses), and total (triangles) tissues of 3-yr-old male (A) and female (B) pike captured at various times from 1976 to 1978. Mean ± 1 SE. Each sampling period is numbered above the abscissa. The values of each parameter (indicated at right by their symbols) were analyzed over the months by a Student–Newman–Keuls test. Sample dates underlined at the same level were not significantly different ($P > 0.05$). Number of animals analyzed for each group are listed across the top of the graph.

from Lac Ste. Anne occurred only from May to September (Fig. 1). The mean lengths of respective age-classes during any particular month were similar for both sexes. There were no significant differences between monthly values from 1976, 1977, or 1978, so all data from the 3 yr were pooled to give a single monthly growth curve. Annuli were completed between May and June. The largest increments in length occurred in June or July, except in young-of-year fish which increased dramatically in length from August through October.

There were no significant differences in body calorific equivalents (4.7 ± 0.1 kcal/g, $\bar{x} \pm 95\%$ CL), percent water ($75.7 \pm 0.2\%$), or kilocalories per ash free gram wet weight (1.5 ± 0.1) between any monthly samples; nor were there any differences between age-class or sex. Total body energy content for all fish was computed by multiplying individual body weights by the mean percent water and calorific equivalent. Water content and calorific equivalents of the liver and gonads varied significantly in different months (Table 1), but did not vary between age-classes during any month. Liver and gonad energy content for the fish were computed by multiplying the respective organ weights by the monthly mean percent water and calorific equivalent for each sex. Total energy content of a fish was considered the sum of body, liver, and gonad energy while somatic energy was considered the sum of body and liver energy.

Depletion of body energy reserves occurred in both sexes during spawning (March to May) (Fig. 2). Body

TABLE 1. Percent water (% W) and calorific (1 kcal = 4186 J) equivalents (kcal/g dry wt) for liver and gonad tissue for pike collected at Lac Ste. Anne from March 1976 to May 1977. $\bar{x} \pm 95\%$ CL (Student–Newman–Keuls test). The analysis was based on 145 mature pike, with a mean of 11 fish per group (range 6–14).

Group	Liver % W	Liver calorific equivalent	Gonad % W	Gonad calorific equivalent
March				
Males	72.1±15.2	5.2±0.2	73.8±2.0	5.5±0.1
Females	78.4±2.0	5.3±0.3	63.3±1.9	5.6±0.2
May				
Males	74.9±1.5	6.4±0.2	77.8±4.1	5.1±0.1
Females	77.8±1.2	6.0±0.2	78.7±6.0	5.1±0.1
August				
Males	66.9±1.2	6.3±0.2	76.2±4.4	4.8±0.1
Females	68.9±2.6	6.3±0.2	82.7±1.4	5.1±0.1
January 77				
Males	71.3±1.0	5.3±0.1	79.0±0.8	5.4±0.1
Females	79.4±0.8	5.2±0.1	65.5±0.7	5.8±0.1
March				
Males	69.7±1.1	5.0±0.2	77.6±1.9	5.4±0.1
Females	78.0±0.6	5.0±0.1	65.2±0.5	5.9±0.1
May				
Males	77.2±1.8	5.6±0.3	83.0±1.9	5.3±0.1
Females	81.9±1.4	5.3±0.2	85.3±2.7	5.1±0.1
Mean				
Males	71.9±0.9	5.7±0.2	78.5±1.4	5.3±0.1
Females	76.8±0.9	5.6±0.2	71.9±1.6	5.3±0.2

energy increased from June to January in 3-yr-old males, while females only increased in body energy over the summer (June–August).

GONAD GROWTH

Some resorption of testis energy occurred from May to August, while little change in ovary energy occurred during this time (Fig. 2). Spawning occurred in late April or early May; only young-of-year fish did not mature. Testicular growth occurred entirely in August of each year. Ovary caloric growth began in August, and nearly all ovarian growth (81%) occurred during the winter (October–March).

Gonad energy content of mature fish was positively correlated with body weight and age for both sexes. However, there was no significant change in the relative gonad energy (gonad kcal/body kcal) with age or weight in either sex.

LIVER GROWTH

Total liver calories of 3-yr-old pike (Fig. 2) decreased during the spring each year, then increased through the summer. The loss of energy from the male liver in August (15.6 kcal) approximately equalled the amount of testis growth (16.8 kcal) during that time. Fifty-three percent of the total liver growth occurred from May to October in males, while all of the female liver growth occurred from May to October.

Liver energy content in both sexes of pike was positively correlated with age and body weight. There was no change in the relative liver energy (liver kcal/body kcal) of either sex with age or weight.

SPAWNING DEPLETION

The causes of depletion were assessed by measuring energy content of tissues from pike captured during the spawning time. During late April, males were readily captured by gillnet, while relatively few females were taken. The results for females are therefore based on a small April sample (five fish). Total liver energy decreased significantly in 2-yr-old males during both the prespawning (March–late April) and spawning (late April–May) periods (Table 2). A significant decrease in body energy occurred only in the prespawning phase. There was no change in testis energy content from March to late April.

Two-year-old females had a significant decrease in total liver energy only in the prespawning period. Somatic (body and liver) energy showed a significant decreasing trend from March to May. Ovary energy content did not change from March to late April.

As much as 68% of the total liver energy was used during spawning depletion in pike, which indicates that the liver is an energy depot. Somatic energy was only depleted by 13%, but this depletion contributed 4.6 fold more energy than the liver losses, indicating that the body is the most important energy store in pike.

TABLE 2. Caloric content (1 kcal = 4186 J) of tissues from 2-yr-old northern pike collected in early March, late April, and early May 1977 and 1978 from Lac Ste. Anne. Symbols listed under range test indicate each sample, and symbols underlined at the same level were not significantly different ($P < 0.05$, Student–Newman–Keuls test).

	Mar.	Late Apr.	May	Range test
		Male		
n	36	27	13	
Body	1165 ± 22	999 ± 21	1037 ± 43	Mar. Apr. May
Liver	45.3 ± 1.9	34.1 ± 1.9	15.2 ± 1.6	Mar. Apr. May
Gonad	17.9 ± 0.9	17.3 ± 1.0	5.5 ± 0.6	Mar. Apr. May
Somatic	1210 ± 23	1033 ± 22	1052 ± 44	Mar. Apr. May
		Female		
n	22	5	9	
Body	1043 ± 25	959 ± 41	956 ± 23	Mar. Apr. May
Liver	28.4 ± 1.2	17.8 ± 2.2	12.7 ± 1.6	Mar. Apr. May
Gonad	268 ± 18.0	320 ± 17.1	6.4 ± 0.7	Mar. Apr. May
Somatic	1071 ± 25	976 ± 43	969 ± 22	Mar. Apr. May

TABLE 3. Total and somatic calories (1 kcal = 4186 J) for each age-class of pike collected from Lac Ste. Anne in March and May. $\bar{x} \pm 95\%$ CL. Production values (Prod) are the differences between means from May and the following March, and the production efficiency (Eff) is the yearly production/initial caloric content of the fish. Values are based on 145 fish collected in March and May, with a mean of 14 fish/group (range 6–36).

Age	Group	May (A)	March (B)	Prod (B–A)	Eff (B–A)/A
			Males		
0	Somatic	0	554 ± 60	554	—
	Total	0	558 ± 63	558	—
1	Somatic	677 ± 81	926 ± 45	249	37
	Total	681 ± 76	945 ± 47	264	39
2	Somatic	814 ± 61	1210 ± 47	396	49
	Total	818 ± 62	1228 ± 48	410	50
3	Somatic	1052 ± 95	1387 ± 65	335	32
	Total	1057 ± 96	1409 ± 67	352	33
4	Somatic	1244 ± 42	1928 ± 180	684	55
	Total	1252 ± 43	1962 ± 182	711	57
			Females		
0	Somatic	0	554 ± 61	554	—
	Total	0	558 ± 63	558	—
1	Somatic	677 ± 81	930 ± 83	253	37
	Total	681 ± 76	1107 ± 102	426	63
2	Somatic	779 ± 70	1071 ± 52	292	37
	Total	784 ± 71	1339 ± 80	555	71
3	Somatic	969 ± 52	1444 ± 70	475	49
	Total	975 ± 52	1880 ± 106	905	93
4	Somatic	1256 ± 53	1722 ± 94	466	37
	Total	1267 ± 53	2176 ± 121	909	72

TOTAL PRODUCTION

Annual production for northern pike from Lac Ste. Anne (Table 3) was calculated as the difference in energy content (either total or somatic) between May and the following March for each age-class. This period did not include the April depletion time. Three-year-old males increased 352 kcal in total (body, liver, and gonad) energy over the year. Thirty-four percent of this increase occurred in the winter when water temperature was <2°C (Fig. 2).

Three-year-old females accumulated 905 kcal in total

energy from May to March (2.6 times the male increase). Growth during the winter accounted for 35% of the total increase (Fig. 2). Caloric growth in both seasons was more than twice as high in females as in males. A significant increase in somatic (body and liver) energy occurred only during the summer in females, while ovary growth occurred only in the winter. There was no concomitant depletion of somatic energy during ovary growth (October to March).

Somatic production (Table 3) was high during the 1st yr of life, when virtually no energy was used for gonad growth. Total production was lowest in the 2nd yr, then increased for both sexes. Total annual production by females was 1.6 and 2.6 times the male level at age 1 and 3, respectively. Male production was maximum at age 4, female at age 3 and 4. Total production and production efficiencies were always higher in females than males, whereas somatic production was similar in both sexes.

Discussion

Significant total energy accumulation occurred in both sexes during winter and summer. In this study, about 35% of pike growth occurred in the winter at a temperature of ~1°C. While other studies have demonstrated energy accumulation over the summer, fish have generally been found to utilize energy stores for metabolism and gonad maturation during winter (Healey 1972; MacKinnon 1972; Craig 1977; Foltz and Norden 1977). Pike continue to feed during winter (Lawler 1965; Keast 1968), although their ration is only ~10% of the summer level (J. S. Diana unpublished data). The winter growth of pike in this study is in contrast to the conclusions of Johnson (1966) who found that immature pike from Lake Windermere would not grow in captivity at winter temperatures. Yellow perch (*Perca flavescens*) in Lac Ste. Anne also grow over winter (Tanasichuk 1978). In northern areas like Lac Ste. Anne, where fish may spend half of their lives at winter temperatures near 1°C, adaptations to the seasonal cycle may have occurred to allow growth at this temperature.

The timing of growth in length for Lac Ste. Anne pike was similar to pike from two English rivers (Mann 1976). The mean lengths of age-classes at the beginning of the growth year were intermediate when compared to a large number of pike populations (Toner and Lawler 1969). Since pike from Lac Ste. Anne show similar timing and magnitude of growth as other populations, their energy dynamics should also be similar. Therefore, one would expect that in other populations of pike significant male somatic growth and female gonad growth would occur in winter. To properly evaluate winter production, one must sample individuals in late winter before the spawning depletion.

Significant depletions of somatic energy stores occurred in both sexes during spawning. Pike fast at this time (mid-April to the end of spawning [Frost 1954]), and this results in some depletion. Motor activity by pike may also be higher at this time than during other periods of the year (Miller 1948). No gonad growth took place from March to May in either sex, yet significant depletion of somatic energy occurred. Therefore, these depletions were mainly due to spawning-related activities and fast, rather than gonad growth.

Depletion of body energy occurred without any change in body calorific equivalents (kcal/g). Because protein and fat have different calorific equivalents (Phillips 1972), depletion of body energy appears to be due to catabolism of whole tissue rather than catabolism of specific constituents which would alter the calorific equivalents. The liver showed significant changes in calorific equivalents during the year, which indicates that specific constituents are preferentially stored or utilized during various seasons. Similar results to these were found by Medford and Mackay (1978) in pike from Lac Ste. Anne. However, Ince and Thorpe (1976) found that English pike preferentially utilized muscle fat and glycogen during 3 mo of forced starvation. The inability of Ince and Thorpe to use entire fish in their analysis, the effect of 3 mo starvation (much longer than in the present study), or a difference between North American and European pike could explain the discrepancy between our results and theirs.

Gonad growth commenced in August for both sexes, and testicular growth was complete by September. Energy for testis growth may have come from endogenous stores in the liver, because both organs showed nearly the same magnitude of caloric changes during that time. These results are further strengthened by the work of Glass et al. (1977) who found that a specific fatty acid was produced by the liver of male pike during summer, and this fatty acid was depleted from the liver and accumulated in the testes of ripe fish. Ovarian growth continued until March, and the energy required for this must have resulted from food intake, because no depletion of somatic energy occurred during this time.

During their 1st yr, pike produce tissue at an extremely rapid rate. With the onset of sexual maturation, somatic production reached a minimum in both sexes. However, older fish had high production efficiencies, and total production was still high at age 4. Only fish through age 4 were captured frequently enough to estimate production, which did not allow an age comparison over many years. However, our data show no decrease in somatic production with age, indicating that the pike were not becoming overwhelmed by reproductive requirements as they grew older.

Acknowledgments

This study was funded by a graduate research assistantship from the University of Alberta to J.S.D., and in part by NRCC Grant No. A6587 to W.C.M. The work was conducted out of the Lac Ste. Anne Fish Research Laboratory, University of Alberta. D. Glass, D. Goulet, C. Haw-

ryshin, B. Medford, T. Miller, D. Pehowich, D. Porter, P. Praegert, R. Tanasichuk, C. Thoresen, and M. Wiegand aided in the field work. D. Beatty, B. Medford, D. Pehowich, R. Tanasichuk, and M. Wiegand reviewed the manuscript. Special thanks are extended to B. Medford, who did much of the early work at Lac Ste. Anne, and B. Diana, who aided in all portions of this research.

CRAIG, J. F. 1977. The body composition of adult perch, *Perca fluviatilis* in Windermere, with reference to seasonal changes and reproduction. J. Anim. Ecol. 46: 617–632.

FOLTZ, J. W., AND C. R. NORDEN. 1977. Seasonal changes in food consumption and energy content of smelt (*Osmerus mordax*) in Lake Michigan. Trans. Am. Fish. Soc. 106: 230–234.

FROST, W. E. 1954. The food of pike, *Esox lucius* L., in Windermere. J. Anim. Ecol. 23: 339–360.

FROST, W. E., AND C. KIPLING. 1959. The determination of the age and growth of pike (*Esox lucius* L.) from scales and opercular bones. J. Cons. Int. Explor. Mer 24: 314–341.

GLASS, R. L., T. P. KRICK, D. L. OLSON, AND R. L. THORSON. 1977. The occurrence and distribution of furan fatty acids in spawning male freshwater fish. Lipids 12: 828–836.

HEALEY, M. C. 1972. Bioenergetics of a sand bogy (*Gobius minutus*) population. J. Fish. Res. Board Can. 29: 187–194.

HUANG, C. T., AND C. P. HICKMAN JR. 1968. Binding of inorganic iodide to the plasma proteins of teleost fishes. J. Fish. Res. Board Can. 25: 1651–1666.

IDLER, D. R., AND I. BITNERS. 1958. Biochemical studies on sockeye salmon during spawning migration. II. Cholesterol, fat, protein, and water in the flesh of standard fish. Can. J. Biochem. Physiol. 36: 793–798.

1960. Biochemical studies on sockeye salmon during spawning migration. IX. Fat, protein and water in the major internal organs and cholesterol in the liver and gonads of the standard fish. J. Fish. Res. Board Can. 17: 113–122.

INCE, B. W., AND A. THORPE. 1976. The effects of starvation and force-feeding on the metabolism of the northern pike, *Esox lucius* L. J. Fish Biol. 8: 79–88.

JOHNSON, L. 1966. Experimental determination of food consumption of pike, *Esox lucius*, for growth and maintenance. J. Fish. Res. Board Can. 23: 1495–1505.

KEAST, A. 1968. Feeding of some Great Lakes fishes at low temperatures. J. Fish. Res. Board Can. 25: 1199–1218.

LAWLER, G. H. 1965. The food of pike, *Esox lucius*, in Heming Lake, Manitoba. J. Fish. Res. Board Can. 22: 1357–1377.

LOVE, R. M. 1970. The chemical biology of fishes. Academic Press, Inc., London. 547 p.

MACKINNON, J. C. 1972. Summer storage of energy and its use for winter metabolism and gonad maturation in American plaice (*Hippoglossoides platessoides*). J. Fish. Res. Board Can. 29: 1749–1759.

MANN, R. H. K. 1976. Observations on the age, growth, reproduction and food of the pike *Esox lucius* (L.) in two rivers in southern England. J. Fish Biol. 8: 179–197.

MEDFORD, B. A., AND W. C. MACKAY. 1978. Protein and lipid content of gonads, liver, and muscle of northern pike (*Esox lucius*) in relation to gonad growth. J. Fish. Res. Board Can. 35: 213–219.

MILLER, R. B. 1948. A note on the movement of pike, *Esox lucius*. Copeia 1948: 62.

NIE, N. H., C. H. HULL, J. G. JENKINS, K. STEINBRENNER, AND D. H. BENT. 1975. Statistical package for the social sciences. McGraw-Hill Book Company, New York, N.Y. 675 p.

PAINE, R. T. 1971. The measurement and application of the calorie to ecological problems. Annu. Rev. Ecol. System. 2: 145–164.

PHILLIPS, A. M. 1972. Calorie and energy requirement, p. 1–28. *In* J. E. Halver [ed.] Fish nutrition. Academic Press, Inc., New York, N.Y.

SHUL'MAN, G. E. 1974. Life cycles of fish. John Wiley and Sons, New York, N.Y. 258 p.

TANASICHUK, R. W. 1978. Characteristics of body and gonad growth of yellow perch, *Perca flavescens*, from Lac Ste. Anne, Alberta. M.Sc. Thesis, Univ. Alberta, Edmonton, Alta. 102 p.

TONER, E. D., AND G. H. LAWLER. 1969. Synopsis of biological data on the pike. FAO Fisheries Synopsis Number 30.

Detritus and Juvenile Salmon Production in the Nanaimo Estuary: I. Production and Feeding Rates of Juvenile Chum Salmon (*Oncorhynchus keta*)

M. C. HEALEY

Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C. V9R 5K6

HEALEY, M. C. 1979. Detritus and juvenile salmon production in the Nanaimo Estuary: I. Production and feeding rates of juvenile chum salmon (*Oncorhynchus keta*). J. Fish. Res. Board Can. 36: 488–496.

Theories of food chain dynamics have important implications for the management of marine resources. As yet, however, there are few empirical studies of the food chain dynamics of resource species against which these theories can be judged. This paper compares the food requirements of juvenile chum salmon (*Oncorhynchus keta*) in the Nanaimo Estuary with the productivity of their principal food species. Chum were present in the estuary from March until June and the estuary population ranged up to 4.1 million in May 1975 and 2.4 million in April 1976. The average weight of chum was 0.66 g in 1975 and 0.65 g in 1976, and their rate of growth averaged ~6% body weight per day in both years. Food intake estimated by three independent methods ranged 4.4–18% body weight per day and was assumed to average 15% body weight per day. Annual fry production was 2381 kg in 1975 and 1122 kg in 1976. Food intake was 6184 kg in 1975 and 2815 kg in 1976. The principal dietary item in both years was harpacticoid copepods. In 1975 *Harpacticus uniremis* made up 50% of the diet overall, and >80% of the diet when fry were most abundant. The seasonal pattern of abundance of fry and *H. uniremis* on the estuary was the same, and the fry consumed most of the estimated production of *H. uniremis*. Juvenile chum production was potentially limited by food supply. Food chain dynamics were, therefore, important in the productivity of the chum population, but since both chum and its chief food were rare and ephemeral elements of the estuarine fauna, their interaction probably had little impact on the dynamics of the estuary as a whole.

Key words: chum salmon, estuaries, food chains, populations, feeding rates

HEALEY, M. C. 1979. Detritus and juvenile salmon production in the Nanaimo Estuary: I. Production and feeding rates of juvenile chum salmon (*Oncorhynchus keta*). J. Fish. Res. Board Can. 36: 488–496.

Les théories sur la dynamique de la chaîne alimentaire ont une grande influence sur la gestion des ressources marines. Cependant, il y a très peu d'études empiriques de la dynamique de la chaîne alimentaire des espèces qui constituent la ressource, études qui permettraient d'évaluer ces théories. Dans le présent article, nous comparons les besoins alimentaires de jeunes saumons keta (*Oncorhynchus keta*) dans l'estuaire de Nanaïmo avec la productivité des principales espèces proies. Il y avait des saumons keta dans l'estuaire de mars à juin, et la population estuarienne atteignait 4,1 millions en mai 1975 et 2,4 millions en avril 1976. Le poids moyen des saumons keta était de 0,66 g en 1975 et 0,65 g en 1976. Ces deux années, le rythme de croissance étaient en moyenne ~6% du poids corporel par jour. La ration alimentaire, estimée selon trois méthodes indépendantes, variait entre 4,4 et 18% du poids corporel par jour, et on a supposé une moyenne de 15% du poids corporel par jour. La production annuelle d'alevins était de 2 381 kg en 1975 et 1 122 kg en 1976. La ration alimentaire était de 6 184 kg en 1975 et 2 815 kg en 1976. Ces deux années, les copépodes harpacticoïdes prédominaient dans le régime alimentaire. En 1975, *Harpacticus uniremis* constituait jusqu'à 50% du régime global et >80% du régime au moment où les alevins étaient à leur maximum d abondance. Le schéma saisonnier d'abondance des alevins et celui de *H. uniremis* dans l'estuaire étaient identiques et les alevins consumèrent la majeure partie de la production estimée de *H. uniremis*. La production de jeunes saumons keta a possiblement été limitée par les quantités de nourriture disponible. La dynamique de la chaîne alimentaire est donc importante dans la productivité de la population de saumons keta. Mais comme le saumon keta ainsi que ses principales proies sont des éléments rares et éphémères de la faune estuarienne, leur interaction a probablement peu d'effet sur la dynamique de l'estuaire en général.

Received May 29, 1978
Accepted January 22, 1979

Reçu le 29 mai 1978
Accepté le 22 janvier 1979

Printed in Canada (J5259)
Imprimé au Canada (J5259)

SEVERAL recent studies have pointed out the importance of food chain relationships to the population dynamics of marine resource species (Parsons et al. 1969; LaBrasseur et al. 1969; Parsons and LeBrasseur 1970; Edwards and Steele 1968; Edwards et al. 1969, 1970; Steele and Edwards 1970; Steele et al. 1970; Walsh 1975). Concurrently there have been advances in the theory of marine food chain dynamics in relation to man as a predator in which trophic relationships are used to predict or optimize yield in a fishery (Dickie 1976; Sheldon et al. 1977; Steele 1974). Furthermore, the importance of the classical oceanic food chain, plant–herbivore–carnivore, is being challenged by an alternative food chain, detritus–microbe–consumer (Pomeroy 1974). The new theories of marine food chain dynamics hold considerable promise for the improved management of ecosystems. As yet, however, there are relatively few detailed studies of the relationship between marine resource species and the ecosystem that supports them. Such studies are essential if the practicality of the new theories is to be determined.

Sibert et al. (1977) reported preliminary results relating the production of an important resource species, chum salmon (*Oncorhynchus keta*), to a detritus-based food web in the Nanaimo Estuary, British Columbia. The purpose of this paper, and two others (Sibert 1979; Naiman and Sibert 1979), is to describe in more detail the ecological basis of juvenile salmon production in the Nanaimo Estuary, and to consider whether food supply is likely to limit salmon production in the estuary. In this paper I describe the abundance, productivity, food habits, and feeding rates of chum salmon in the estuary. Sibert (1979) and Naiman and Sibert (1979) deal with populations of food organisms and the importance of detritus in fish food production.

Study Area

The Nanaimo River is a 6th order stream on the east coast of Vancouver Island and drains a watershed of 894 km^2. River discharge averages 41 m^3/s annually, ranges 3–148 m^3/s monthly, and is dominated by winter freshets. Chum, coho (*O. kisutch*), and chinook (*O. tshawytscha*) salmon spawn in the river. Both chum and chinook fry utilize the estuary as a nursery area, but chum are two orders of magnitude more abundant.

The delta–estuary area of the river occupies ~8.6 km^2 of which ~6.5 km^2 is intertidal. The delta is formed by sedimentation between the mainland shore and a long rocky peninsula (Fig. 1). The river divides into two main channels which cross the intertidal sandflat on the east and west sides. Numerous small stream channels cross the sandflat between the two main channels. The estuary borders on the harbor of Nanaimo city and is subject to some dredging along its active delta front. The intertidal zone is used for temporary storage of log rafts by local saw mills and a pulp mill. Log storage leases cover about 30% of the intertidal area but are not fully utilized. Some diking to create farmland has occurred along the southern margin of the delta.

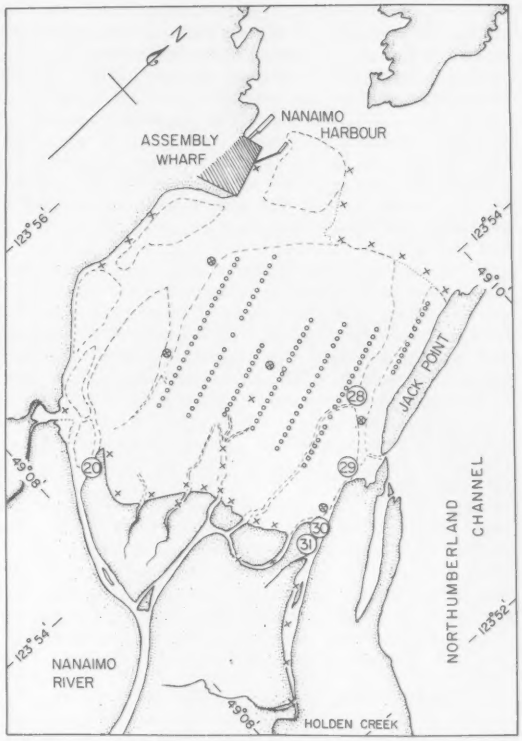


FIG. 1. The Nanaimo Estuary showing location of fry traps in the river (20); location of regular sampling sites on the east side of the estuary (28), (29), (30), (31); location of sites sampled by beach seine to determine fry distribution, X; and sites sampled by purse seine at high tide (X).

Although the river influence extends well beyond the delta front, the data presented here refer to the intertidal area and the immediately adjacent waters.

Adult chum salmon enter the Nanaimo River mainly in October and November and spawn in the lower 8 km of the river. Since 1949, the spawning escapement has averaged about 38 000 (Aro 1973). From March to May chum fry emerge from the gravel of the spawning beds and migrate to sea. In the sea most fry occupy shallow water close to shore for several weeks, feeding on benthic and epibenthic organisms. The estuary of the Nanaimo River is a major nursery area for chum fry during their first few weeks of ocean life. Later, the fry move offshore and adopt a pelagic, plankton-feeding existence.

Methods

The downstream run of chum fry was monitored in 1975 and 1976 by fry trapping near the river mouth (Fig. 1). The total fry run each day was estimated by mark and recapture techniques in 1975 (Healey et al. 1977a). Total daily run in 1976 was estimated from trap efficiency determined in 1975. In both 1975 and 1976, segments of the

run were marked with different colors of fluorescent grit, so that different parts of the run could be identified on the estuary (Healey et al. 1976). The distribution and abundance of chum fry in the estuary was monitored by weekly beach seining at low tide along the river channel and slough on the east side of the delta (Fig. 1). Distribution of fry was determined by irregular beach seining in other locations at low tide, and along the southern margin of the delta at high tide. In 1976 a series of sets was made among the log booms at high tide with a small purse seine to determine whether fry remained out over the sandflat at high tide (Fig. 1).

Up to 2000 fry were preserved from each weekly sampling of the east channel to be examined for marked fry. All marked fry plus a subsample of 20 or more unmarked fry were further analyzed for fork length, preserved weight, and stomach contents. Fish captured at high tide from the southern margin of the delta were preserved for comparison of high and low tide stomach contents in 1975.

In 1975 the wet weights of the stomach contents of individual fry were recorded. In 1976 dry weights of grouped stomach contents of 10–20 fry were recorded rather than individual weights. This change in procedure was made because replicate subsamples of 10–20 stomachs from the same sample were found to differ by up to 35% when individual wet weights were measured, compared with 15% when grouped dry weights were measured. In 1975 the stomach contents were sorted into six size categories, and into major taxonomic groups. The number of organisms in each size category and each taxon was recorded. Harpacticoid copepods were a dominant food item and the harpacticoids in the grouped stomach contents of fry from seven different samples were identified to species. Detailed analysis of stomach contents was not done in 1976; however, the dominant food item in the stomachs was noted.

The length of residence of the marked fry on the estuary was estimated as the difference between the weighted mean release date for each marked cohort in the river, and the weighted mean recapture date for each cohort in the estuary:

$$D = \sum^{d} dR_d / \sum^{d} R_d \tag{1}$$

$$T = D_r - D_e \tag{2}$$

where D = the weighted mean release or recapture day for each mark
d = the Julian day of release or recapture
R_d = the marked fry released or recaptured (expressed as a catch-per-unit-effort) on day d
D_r and D_e = the mean release and recapture dates, respectively, for a marked cohort
T = the average residence time in days

The population of chum in the estuary was estimated from the daily downstream run of fry, and their residence in the estuary as determined above. Each daily input of fry to the estuary was treated as a separate cohort. The rate of disappearance of that cohort from the estuary was assumed to be constant and determined by the residence time for the cohort. Alternative hypotheses involving a variable rate of disappearance gave poorer fits to the empirical data. The residence time for the cohort was taken to be the same as the residence time for the marked fry in the cohort. The estuary population on any day was taken as the sum of the members of all previous daily cohorts remaining in the estuary:

$$N_t = N_o e^{-rt} \tag{3}$$

$$r = \log_e(N_o)/T \tag{4}$$

$$P_n = \sum_{t=1}^{n} N_{t_n} \tag{5}$$

where N_o = the number of fry entering the estuary on any day (equal of the downstream run for that day)
N_t = the number of fry from that cohort remaining on the estuary t days after entering the estuary
r = the rate of disappearance of the cohort from the estuary
T = the residence time for the cohort in days
P_n = the estuary population estimate for day n
N_{t_n} = the number of fry entering the estuary on day i that still remain in the estuary on day n

Confidence limits on the estimate of the daily downstream run of fry in 1975 were set by treating the ratio of marks recaptured in the fry traps to marks released upstream (i.e. trap efficiency) as a binomial variable. The normal approximation to the binomial was used in setting these limits. Limits on the total estimated fry run for 1976 were set by assuming that the error variance was the same as in 1975. Upper and lower 95% confidence limits for the fry run were used to establish minimum upper and lower estimates for the estuary fry population. These are not 95% confidence limits for the estuary population since sources of error in the estimate of residence time are not accounted for. The upper and lower estimates are presented merely to establish minimum boundaries on the subsequent estimates of feeding rate.

Growth rates of fry were estimated from the weights of recaptured marked fry, assuming exponential growth in weight. Recaptures from the estuary were combined with recaptures from other locations in these estimates. The inclusion of recaptures from other locations was necessary because the larger fish tended to leave the estuary earlier than smaller fish, so that growth rates determined from estuary recaptures alone were biased low (Healey et al. 1977b).

Biomass of chum on the estuary each day was estimated as the product of the daily population estimate and the average weight of fry sampled from the estuary in that calendar week. Production was estimated as the product of the daily biomass and the instantaneous growth in weight of the fry summed over the period of the study.

Food requirements were estimated from literature values of gross conversion efficiency of chum fry fed natural foods (LeBrasseur 1969), from bioenergetic considerations (Winberg 1956), and from rates of gastric evacuation (Kitchell et al. 1974).

Results

The timing of the downstream run of chum fry was similar in 1975 and 1976 although the peak of the run was earlier in 1976. Fry movement occurred from early March until early June with maximum movement from mid-April to mid-May (Fig. 2). In 1975 an estimated 52.4 million fry moved downstream (95% confidence limits 39.7–85.3 million) and in 1976 the estimate was

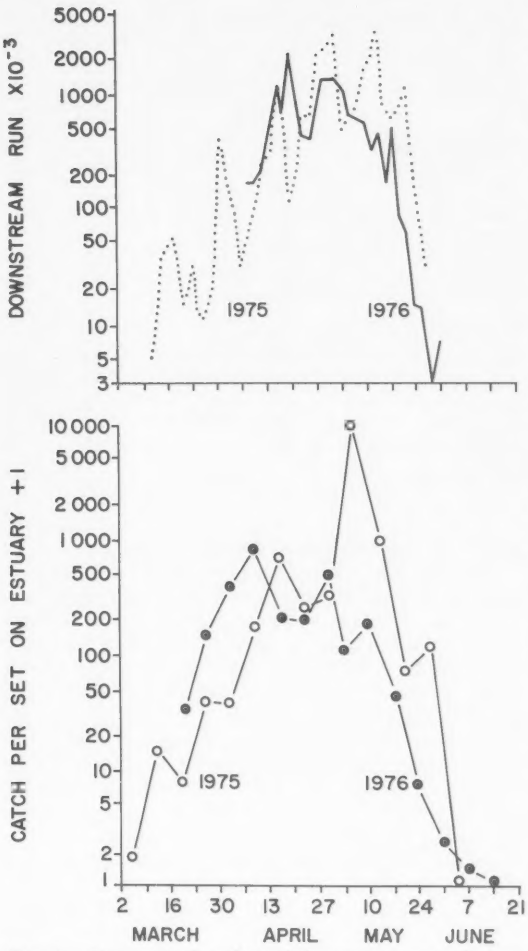


FIG. 2. *Upper panel*, estimated downstream run of chum fry in 1975 and 1976; *lower panel*, catch per beach seine set on the east side of the estuary at low tide in 1975 and 1976.

29.6 million (95% confidence limits 22.4–48.2 million). The seasonal pattern of fry abundance on the estuary was also similar in 1975 and 1976 (Fig. 2). Fry were present in the estuary from early March until early July. Numbers peaked in the estuary somewhat earlier in 1976, consistent with the earlier peak in downstream run. Fry were also captured in the estuary later in 1976. This occurred because samples were taken further seaward along the channel on the east side of the delta in 1976. Stragglers remained in the lower reaches of the channel for some time after most of the fry had left the estuary.

At high tide the fry occurred in schools scattered across the southern margin of the delta and were not found in the open water over the tidal flats or among the log booms. At low tide the fry retreated into the tidal creeks and stream channels that retained water at low tide. The east channel of the river and the slough along the east side of the estuary were particularly favored refuges at low tide and fry were rare in stream channels on the west side of the estuary. The characteristics of the favored refuges on the east side of the estuary included quiet backwaters adjacent to deeper water, sandy sediments, and the presence of eelgrass (*Zostera* sp.).

TABLE 1. Residence time (days) of different fractions of the chum run in the Nanaimo Estuary in 1975 and 1976.

1975		1976	
Cumulative % of run	Days residence	Cumulative % of run	Days residence
0.7	14.6	31.5	9.3
2.3	14.9	69.4	4.5
3.8	17.1	100	0.0
13.8	18.5		
100	1.8		

Both the timing and the size of the run were misjudged in 1975 so that the different marks were not well distributed throughout the run. Approximately equal distribution of the three marks was achieved in 1976, however (Table 1). Mark recaptures indicated that fry from the early part of the run spent considerably longer in the estuary than fry from the latter part of the run. Estimated residence times ranged from >18 to <2 d in 1975 and from about 9 to 0 in 1976 (Table 1).

Estimates of the estuary population for 1975 and 1976 from equation (5) produced patterns of abundance that reflected the seasonal abundance pattern from beach seining (Fig. 3). In 1975, the maximum estimated estuary population was 4.1 million and in 1976 it was 2.4 million. Sampling in 1976 began after the downstream run had been under way for some time. In calculating total estuary biomass and production, I assumed that the estuary population of fry prior to April 7 in 1976 was the same as in 1975. Although the run was smaller in 1976 it was also earlier. The assumption of similar population densities prior to April 7, therefore, seemed most appropriate. This correction increased the initial production estimate for 1976 by ~10%.

Downstream migrant chum fry averaged 0.41 g wet weight. Mean weight of fry sampled from the estuary ranged 0.46–1.02 g in 1975 and 0.35–1.20 g in 1976. During the peak of the population on the estuary average weight of fry ranged between 0.5 and 1 g, and the fish averaged slightly heavier in 1975 than in 1976 (Table 2). The low average weight of fry on the estuary and the absence of any systematic increase in average weight with season reflected the relatively short

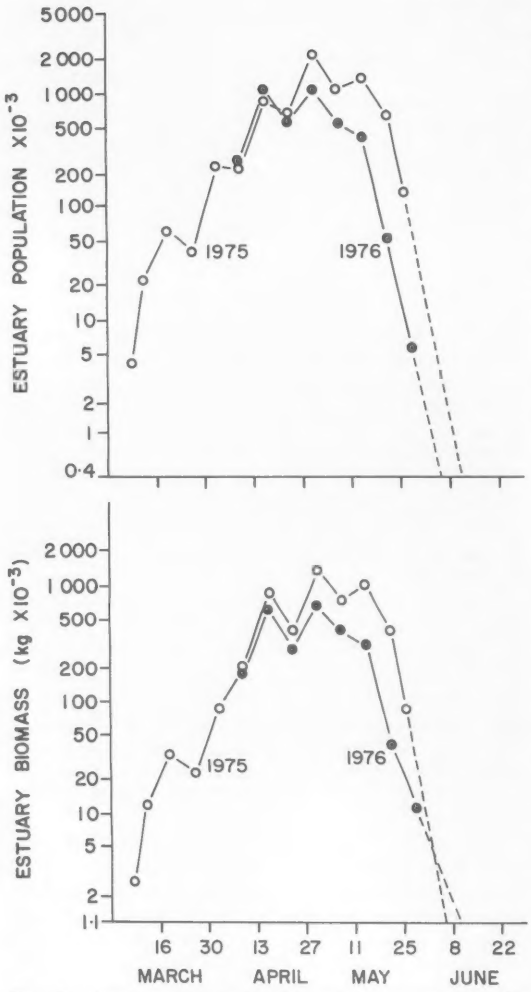


FIG. 3. *Upper panel*, estimated population of chum fry in the estuary in 1975 and 1976; *lower panel*, estimated biomass of chum fry in the estuary in 1975 and 1976.

residence time of the fry in the estuary and the continuous recruitment of new fry to the estuary population. The seasonal curves of biomass for the estuary were similar to the curves of population number (Fig. 3). Maximum estimated biomass of juvenile chum in the estuary was 2400 kg in 1975 and 1200 kg in 1976.

There were no significant differences in growth in weight of different marked cohorts of fry in either 1975 or 1976, although fry from the middle part of the run tended to grow fastest. The rate of increase in weight was ~6%/d for both years (Fig. 4). Only recaptures from the first two mark releases were included in the 1976 estimate since none of the fry from the third mark release were recaptured in the estuary.

TABLE 2. Mean weight in grams ($\bar{x}$) of chum fry captured in the Nanaimo River Estuary in 1975 and 1976. n = number of fry measured.

	1975		1976	
Sampling week	n	$\bar{x}$ g	n	$\bar{x}$ g
Mar. 9–15	30	0.46	0	—
Mar. 16–22	39	0.58	38	0.35
Mar. 23–29	35	0.52	0	—
Mar. 30–Apr. 5	382	0.51	70	0.49
Apr. 6–12	168	0.78	67	0.72
Apr. 13–19	230	1.02	75	0.52
Apr. 20–26	73	0.63	74	0.50
Apr. 27–May 3	53	0.57	101	0.49
May 4–10	86	0.81	89	0.65
May 11–17	82	0.69	82	0.67
May 18–24	50	0.67	71	0.71
May 25–31	50	0.67	70	0.80
June 1–7	0		44	1.29
Overall mean		0.66		0.65

Total production of chum fry in the estuary (the product of average biomass and instantaneous growth rate) was 2400 kg in 1975 and 1100 kg in 1976 (Table 3). Maximum daily production was 136 kg in 1975 and 72 kg in 1976. For the intertidal area of the estuary total production amounted to only 0.37 g/m² in 1975 and 0.17 g/m² in 1976.

Fry were observed feeding during both high- and low-tide sampling. Stomach contents of the weekly samples averaged 1.14–5.65% of body weight in 1975 and 1.15–3.85% in 1976 (Table 4). Average values were more variable in 1975, probably reflecting the technique used that year. Individual stomach contents did not exceed 8% of body weight in 1975. Stomach contents appear to have been greater in 1975 and both years showed a drop in average stomach contents when fry were most abundant on the estuary (Table 4). This suggests that, although food was more abundant in

TABLE 3. Summary of abundance, growth, production, and feeding rates of chum fry on the Nanaimo Estuary.

Year	1975	1976
Downstream migrants	52.4×10^6	29.6×10^6
Upper limit–Lower limit	$85.3–39.7\times10^6$	$48.2–22.4\times10^6$
Cumulative estuary biomass (kg)	41 000	19 000
Upper limit–Lower limit	66 000–32 000	30 000–14 000
Mean daily growth (exp.)	0.058	0.060
% body wt/d	6.0	6.2
Total production (kg)	2 400	1 100
Upper limit–Lower limit	3 000–1 800	1 800–900
Daily ingestion rate (%)	15	15
Total food required (kg)	6 200	2 800
Upper limit–Lower limit	9 900–4 800	4 500–2 200

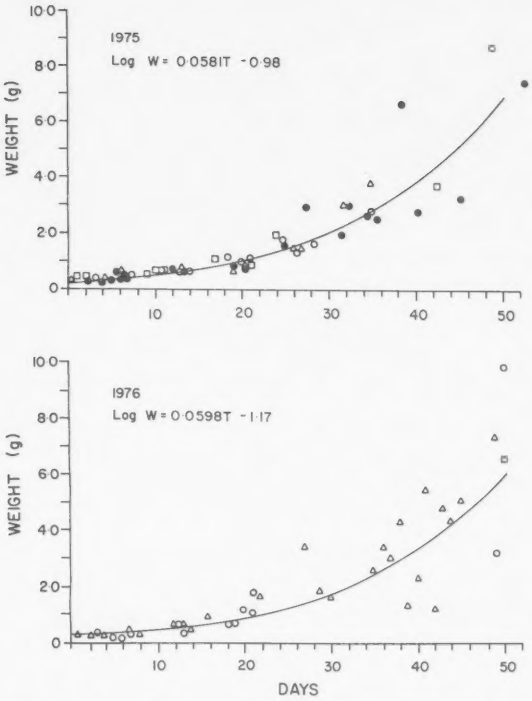


FIG. 4. Growth in weight of color-marked fry in 1975 and 1976. ●, red marks; △, green marks; ○, green–orange marks; X, orange marks. Days is days since weighted mean release day of color mark.

TABLE 4. Average weight of stomach contents as a percent of body weight for chum captured in the estuary in 1975 and 1976. n = number of fry measured.

Sampling week	1975		1976	
	n	%	n	%
Mar. 9–15	30	2.06	—	—
Mar. 16–22	39	4.39	19	2.35
Mar. 23–29	35	1.76	—	—
Mar. 30–Apr. 5	382	5.28	20	1.60
Apr. 6–12	168	2.71	20	1.75
Apr. 13–19	230	2.39	20	1.15
Apr. 20–26	73	1.14	20	1.45
Apr. 27–May 3	56	3.84	20	1.35
May 4–10	86	5.65	20	2.90
May 11–17	84	2.17	20	2.40
May 18–24	—	—	20	3.85
May 25–31	—	—	20	3.20

1975, there was food shortage at peak population densities in both years.

Stomach contents were analyzed in detail only in 1975, but observations made in 1976 suggested that

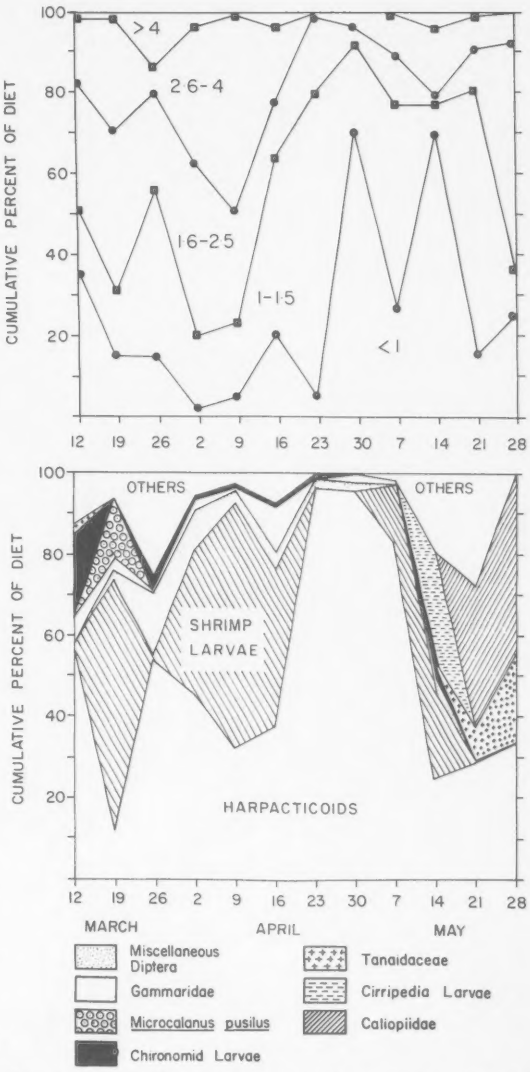


FIG. 5. Size (total length mm) and taxonomic composition of chum diet in 1975.

the food habits of chum did not change. Diet composition from samples taken at high and low tide was similar. Organisms >2.5 mm long were common in the stomach contents of chum only during March and early April. While chum were most abundant on the estuary (April, May) organisms <1.5 mm long and often <1 mm long made up the major numerical proportion of their diet (Fig. 5). Of the major taxa involved, harpacticoid copepods were by far the most numerous, followed by shrimp larvae. Shrimp larvae were important mainly early in the season, however, before fry

became abundant on the estuary. During the peak of the fry population, harpacticoid copepods made up >80% of the diet of chum fry (Fig. 5). Fifteen different species or species complexes of harpacticoid were identified from stomach samples. The samples selected for taxonomic analysis were those in which harpacticoids were most abundant, so that all the important harpacticoid species in the diet were probably identified. *Harpacticus uniremis* was the dominant copepod in all but one of the seven samples examined and far outnumbered any other species. Other species that were common in the diet were (in order of importance) *Microarthridion littorale*, *Tisbe* sp., and *Huntemannia jadensis*. The remaining 11 species were represented by only a few specimens.

A growth rate of 6% body weight/d is near the maximum observed for chum fry in captivity and implies good feeding conditions. LeBrasseur's (1969) feeding and growth experiments suggest that such growth would require a ration of at least 18% body weight/d.

Fry in the estuary averaged 0.66 g wet weight and 4.2 cm fork length during April and May (Table 2). With each tidal cycle the fry retreated into tidal creeks at low tide, and dispersed along the southern margin of the estuary at high tide. The distance involved in each tidal migration was on the order of 2000 m, or 4000 m/d. To cover this distance the fry had to swim an average of about 1 body length/s. If additional travel during feeding occurred, the average swimming speed might have been as high as 2 lengths/s. Observations on fry schools in the estuary suggested that sustained rates of swimming were unlikely to exceed two lengths per second. Brett (1964) gives equations relating oxygen consumption to temperature and swimming speed for young sockeye salmon (*O. nerka*). Chum fry probably have similar rates of metabolism. Estuary temperature varied diurnally, seasonally, and with tide, but for most of April and May was probably in the range of 10–15°C (Brown et al. 1976; Healey unpublished data). These data permit estimates of the average active metabolic rate of chum, which can be combined with growth to generate estimates of daily ration from Winberg's (1956) balanced equation:

$$R = (G + M)/p \tag{6}$$

where R = daily ration in calories
G = daily growth in calories
M = daily metabolism in calories
p = the proportion of the daily ration that is available for growth and metabolism, assumed ≅0.8

The metabolic rate of a 0.66-g salmon at 10°C and a swimming speed of 1 length/s is 1.58 mg O_2/d (Brett 1964), or about 5.5 cal/d (1.31 J/d). For the same fish at 15°C and a swimming speed of 2 lengths/s, metabolic rate is 3.88 mg O_2/d or about 31.6 cal/d (7.55 J/d). Growth at 6%/d is 39.6 mg for a 0.66-g fish, or about 39.6 cal (9.46 J). Daily ration (R) is, therefore, 56.4 cal (13.5 J) at 10°C and 1 length/s, or 66.5 cal (15.9 J) at 15°C and 2 lengths/s. This is equivalent to 8.5–10.1% body weight per day, or about half that predicted from LeBrasseur's results.

Stomach contents during April and May averaged only 3.3% of body weight in 1975 and 2.2% in 1976 (Table 4). Estimates of gastric evacuation for chum (Healey unpublished data) were consistent with those observed for other salmonids (Windell and Norris 1969; Brett and Higgs 1970; Elliott 1972). At 10 and 15° salmonids would be expected to evacuate their stomach contents about 2 and 4 times a day. These gastric turnover rates suggest daily rations of 6.6–13.2% body weight per day in 1975 and 4.4–8.8%/d in 1976, values comparable to estimates based on bioenergetics.

Although estimates of daily ration based on bioenergetics and gastric evacuation were comparable, I believe these values are too low. Rates of active metabolism were calculated from equations for fish 30–60 g in weight and the metabolism per unit weight of fish <1 g is likely to be larger. The metabolic rates also refer to fasting fish, and the metabolism of feeding fish is probably higher. Published rates of gastric evacuation are for single meals fed to fasting fish, and may, therefore, underestimate rates of gastric evacuation in continuously feeding fish. Finally, an ingestion rate of 10% body weight or less seems too low to support a growth rate of 6%/d in consideration of the widely published values of growth efficiency in fishes. An ingestion rate of 18%/d, on the other hand, seems too high considering the low average weight of food in the fishs' stomachs. The growth efficiency of fish is quite variable, and under the right physiological circumstances fish appear to be able to direct a high proportion of ingested energy into growth (Paloheimo and Dickie 1966; Healey 1972). The true ingestion rate is probably ~10–18% body weight per day. For estimating total food consumption on the estuary I used a ration of 15% body weight per day. This value may still be conservatively high.

At a feeding rate of 15%/d, the total food requirement to support the estuary population of chum was 6200 kg wet weight in 1975 and 2800 kg in 1976 (Table 3). Limits on these estimates, based on the upper and lower limits for the estuary population of chum are 4800–9900 kg for 1975 and 2200–4500 kg for 1976 (Table 3). These values represent an average consumption of 0.95 g/m² in 1975 and 0.43 g/m² in 1976 for the intertidal area as a whole.

During 1975 harpacticoids accounted for ~62% by number of the food organisms consumed by young chum (Fig. 5), and the proportion was probably similar in 1976. Some of the other organisms eaten were larger on average than the harpacticoids, so, by weight, harpacticoids probably contributed ~50% to the diet. Most of this was *H. uniremis*. The next most important

single group was shrimp larvae which made up 13% of the diet by number and perhaps 20% by weight. Approximately 3100 kg of harpacticoids were consumed by the fry in 1975, therefore, and 1400 kg in 1976, representing 0.48 g/m^2 of intertidal area in 1975 and 0.22 g/m^2 in 1976.

Discussion

Several pieces of evidence suggest that the productivity of chum fry in the Nanaimo Estuary was limited by food supply. Feeding conditions appeared to be better in 1975, as evidenced by the greater amount of food in the stomachs of the fry and their larger average size. At the same time the estuary supported a larger population of fry in 1975 than in 1976, and the fry spent longer on average in the estuary in 1975, possibly in response to the better feeding conditions. In both 1975 and 1976 food intake appeared to be lowest when fry were most abundant in the estuary. In 1975 feeding was concentrated on *H. uniremis*, a comparatively rare member of the epibenthic fauna. A very high proportion of the annual production of this species was taken by the fry in 1975, and the seasonal pattern of abundance of fry in the estuary was the same as the seasonal pattern of abundance of this preferred food (Sibert 1979). This suggests that alternative food resources were not available to the fry, and that their residence and productivity in the estuary was somehow tied to *H. uniremis*. The similar growth rates of fry in both years is not inconsistent with the hypothesis of food limitation, since the growth rates reported are for the Nanaimo area as a whole, not the estuary alone. Information on stomach contents from other nursery areas in 1975 and 1976 suggests that feeding conditions were similar for the area as a whole in both years (Healey unpublished data).

The high proportion of *H. uniremis* production taken by the fry raises the question of availability of this species to the fry. The distribution of fry in the estuary was not closely related to the distribution of *H. uniremis* (Sibert 1979). Most of the fry occurred on the east side of the estuary, so their exploitation of *H. uniremis* could not have been uniform, although their twice daily migration up and down the estuary with the tide probably brought them into contact with most of the intertidal area on the east side of the estuary. The presence of *H. uniremis* on the west side, but the absence of fry from this area at low tide, suggests that the presence of the preferred food alone was not sufficient to attract the fry. Either the habitat configuration on the west side made the *H. uniremis* unavailable, or the stream channels were otherwise unsuitable as a low tide refuge. The fact that *H. uniremis* was not uniformly available to the fry, whatever the reason, strengthens the suggestion of food limitation, but further suggests that either habitat characteristics or the behavior of *H. uniremis* made this prey very available to the fry.

Although it appears from these results that food limitation occurs in the Nanaimo Estuary, there are enough uncertainties in the data that this conclusion must be regarded with caution. The estimate of downstream run applies to the point of release of the marked fry, which was well upstream from the fry traps. Mortality occurring between the release site and the estuary could not be corrected for, and it is possible that such mortality is quite high (Hunter 1959). The estimate of estuary population size may, therefore, be too high. Mortality of fry may be biased toward the smallest size groups (Parker 1971; Sibert and Parker 1972), resulting in an overestimate of both growth rate and daily ration. Any upward bias in growth rate due to size selective mortality would be offset by the tendency of larger chum to disperse from the study area. Errors in either the instantaneous growth rate of the fry or the estimate of estuary population would have to be very large to alter the conclusion of food limitation, however, since these contribute only a portion of the evidence in support of the conclusion.

The Nanaimo Estuary is not unique in the matter of juvenile salmon preying upon epibenthic food resources. This relationship is well documented for other species and other areas (Kaczynski et al. 1973; Feller and Kaczynski 1975; Mason 1974). Chum fry using other nearshore nursery areas in the Nanaimo area also fed on epibenthic prey and *H. uniremis* in particular (Healey unpublished data). Results from the Nanaimo Estuary are probably generally applicable to many other estuarine and nonestuarine nursery areas of salmon fry. The first few weeks after salmon fry enter the sea is a period of high mortality (Parker 1965) and rapid growth rate of fry has been considered a mechanism reducing mortality by predation (Parker 1971). Food limitation at this time could be critical to production in salmon stocks.

The relationship between juvenile chum salmon and *H. uniremis* in the Nanaimo Estuary is one of a very strong interaction between two relatively rare and ephemeral elements in the ecosystem. The food chain supporting these species is of the detritus–microbe–consumer type (Sibert 1979; Naiman and Sibert 1979), and the Nanaimo Estuary appears to be dominated by this food chain. Yet, the interaction between chum salmon and their epibenthic prey probably has an infinitesimal impact on the dynamics of the estuary as a whole. One should not expect, therefore, that the important features of the ecosystem, from the point of view of the salmon, could satisfactorily be reflected in any general or holistic hypothesis of the dynamics of estuarine ecosystems as proposed by Mann (1975). In the Nanaimo and probably in other estuaries continued production of salmon depends upon the conservation of specific food resources and the habitat characteristics that make these resources available to the salmon, rather than preservation of any overall configuration or dynamic process of the estuary.

Acknowledgments

Technical assistance throughout this study was provided by R. V. Schmidt, F. P. Jordan, R. M. Hungar, and A. C. Phillips. Fry trapping in the lower Nanaimo River was performed by Mr R. Wilson under contract. Dr J. R. Sibert, Dr R. J. Naiman, Dr P. A. Breen, and Dr J. C. Mason critically reviewed drafts of the manuscript.

ARO, K. V. 1973. Salmon and migratory trout of the Nanaimo River and adjacent streams (revised 1973). Fish. Res. Board Can. MS Rep. 1284: 15 p.

BRETT, J. R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Board Can. 21: 1183–1226.

BRETT, J. R., AND D. A. HIGGS. 1970. Effect of temperature on the rate of gastric digestion in fingerling sockeye salmon, *Oncorhynchus nerka*. J. Fish. Res. Board Can. 27: 1767–1779.

BROWN, T. J., J. SIBERT, AND K. STEPHENS. 1976. Data pertaining to the lower trophic levels of Nanaimo River estuary and adjacent waters from March 1974 to March 1975. Fish. Mar. Serv. Data. Rec. 20: 146 p.

DICKIE, L. M. 1976. Predation, yield, and ecological efficiency in aquatic food chains. J. Fish. Res. Board Can. 33: 313–316.

EDWARDS, R., AND J. H. STEELE. 1968. The ecology of O-group plaice and common dabs at Loch Ewe. I. Population and food. J. Exp. Mar. Biol. Ecol. 2: 215–238.

EDWARDS, R. R. C., D. M. FINLAYSON, AND J. H. STEELE. 1969. The ecology of O-group plaice and common dabs at Loch Ewe. II. Experimental studies of metabolism. J. Exp. Mar. Biol. Ecol. 3: 1–17.

EDWARDS, R. R. C., J. H. STEELE, AND A. TREVALLION. 1970. The ecology of O-group plaice and common dabs at Loch Ewe. III. Prey–predator experiments with plaice. J. Exp. Mar. Biol. Ecol. 4: 156–173.

ELLIOTT, J. M. 1972. Rates of gastric evacuation in brown trout, *Salmo trutta* L. Freshwater Biol. 2: 1–18.

FELLER, R. J., AND V. W. KACZYNSKI. 1975. Size selective predation by juvenile chum salmon (*Oncorhynchus keta*) on epibenthic prey in Puget Sound. J. Fish. Res. Board Can. 32: 1419–1429.

HEALEY, M. C. 1972. Bioenergetics of a sand goby (*Gobius minutus*) population. J. Fish. Res. Board Can. 29: 187–194.

HEALEY, M. C., F. P. JORDAN, AND R. M. HUNGAR. 1976. Laboratory and field evaluating of fluorescent grit as a marking material for juvenile salmonids. Fish. Res. Board Can. MS Rep. 1392: 17 p.

HEALEY, M. C., R. V. SCHMIDT, F. P. JORDAN, AND R. M. HUNGAR. 1977a. Young salmon in the Nanaimo area 1975: 1. Distribution and abundance. Fish. Res. Board Can. MS Rep. 1369: 161 p.

1977b. Juvenile salmon in the Nanaimo area 1975: 2. Length, weight, and growth. Fish. Mar. Serv. MS Rep. 1438: 147 p.

HUNTER, J. G. 1959. Survival and production of pink and chum salmon in a coastal stream. J. Fish. Res. Board Can. 16: 835–886.

KACZYNSKI, V. W., R. J. FELLER, AND J. CLAYTON. 1973. Trophic analysis of juvenile pink and chum salmon (*Oncorhynchus gorbuscha* and *O. keta*) in Puget Sound. J. Fish. Res. Board Can. 30: 1003–1008.

KITCHELL, J. F., J. F. KOONCE, R. V. O'NEILL, H. H. SHUGART JR., J. J. MAGNUSON, AND R. S. BOOTH. 1974. Model of fish biomass dynamics. Trans. Am. Fish. Soc. 103: 786–798.

LEBRASSEUR, R. J. 1969. Growth of juvenile chum salmon (*Oncorhynchus keta*) under different feeding regimes. J. Fish. Res. Board Can. 26: 1631–1645.

LEBRASSEUR, R. J., W. E. BARRACLOUGH, O. D. KENNEDY, AND T. R. PARSONS. 1969. Production studies in the Strait of Georgia. Part III. Observations on the food of larval and juvenile fish in the Fraser River plume, February to May 1967. J. Exp. Mar. Biol. Ecol. 3: 51–61.

MANN, K. H. 1975. Relationship between morphometry and biological functioning in three coastal inlets of Nova Scotia, p. 634–644. *In* L. E. Cronin [ed.] Estuarine research, Vol. 1. Academic Press Inc., New York, N.Y.

MASON, J. C. 1974. Behavioural ecology of chum salmon fry (*Oncorhynchus keta*) in a small estuary. J. Fish. Res. Board Can. 31: 83–92.

NAIMAN, R. J., AND J. R. SIBERT. 1979. Detritus and juvenile salmon production in the Nanaimo Estuary: III. Importance of detrital carbon to the estuarine ecosystem. J. Fish. Res. Board Can. 36: 504–520.

PALOHEIMO, J. E., AND L. M. DICKIE. 1966. Food and growth of fishes. III. Relations among food, body size and growth efficiency. J. Fish. Res. Board Can. 23: 1209–1248.

PARKER, R. R. 1965. Estimation of sea mortality rates for the 1961 brood year pink salmon on the Bella Coola area, British Columbia. J. Fish. Res. Board Can. 22: 1523–1554.

1971. Size selective predation among juvenile salmonid fishes in a British Columbia inlet. J. Fish. Res. Board Can. 28: 1503–1510.

PARSONS, T. R., AND R. J. LEBRASSEUR. 1970. The availability of food to different trophic levels in the marine food chain, p. 325–343. *In* J. H. Steele [ed.] Marine food chains. Oliver and Boyd, Edinburgh.

PARSONS, T. R., K. STEPHENS, R. J. LEBRASSEUR. 1969. Production studies in the Strait of Georgia. Part I. Primary production under the Fraser River plume, February to May 1967. J. Exp. Mar. Biol. Ecol. 3: 27–38.

POMEROY, L. R. 1974. The ocean's food web, a changing paradigm. BioScience 24: 499–504.

SHELDON, R. W., W. W. SUTCLIFFE JR., AND M. A. PARANJAPE. 1977. Structure of pelagic food chain and relationship between plankton and fish production. J. Fish. Res. Board Can. 34: 2344–2353.

SIBERT, J. R. 1979. Detritus and juvenile salmon production in the Nanaimo Estuary: II. Meiofauna available as food to juvenile chum salmon. J. Fish. Res. Board Can. 36: 497–503.

SIBERT, J., T. J. BROWN, M. C. HEALEY, B. A. KASK, AND R. J. NAIMAN. 1977. Detritus based food webs: Exploitation by juvenile chum salmon (*Oncorhynchus keta*). Science 196: 649–650.

SIBERT, J., AND R. R. PARKER. 1972. A model of juvenile pink salmon growth in the estuary. Fish. Res. Board Can. Tech. Rep. 321: 62 p.

STEELE, J. H. 1974. The structure of marine ecosystems. Harvard University Press, Cambridge, Mass. 128 p.

STEELE, J. H., AND R. R. C. EDWARDS. 1970. The ecology of O-group plaice and common dabs in Loch Ewe. IV. Dynamics of the plaice and dab populations. J. Exp. Mar. Biol. Ecol. 4: 174–187.

STEELE, J. H., A. D. MCINTYRE, R. R. C. EDWARDS, AND A. TREVALLION. 1970. Interrelations of a young plaice population with its invertebrate food supply, p. 375–388. *In* A. Watson [ed.] Animal populations in relation to their food resources. British Ecological Society Symposium No. 10. Blackwell, Oxford.

WALSH, J. J. 1975. A spatial simulation model of the Peru upwelling ecosystem. Deep Sea Res. 22: 201–236.

WINBERG, G. G. 1956. Rate of metabolism and food requirements of fishes. Fish. Res. Board Can. Transl. Ser. No. 194 (1960): 201 p.

WINDELL, J. T., AND D. C. NORRIS. 1969. Gastric digestion and evacuation in rainbow trout. Prog. Fish. Cult. 31: 20–26.

Detritus and Juvenile Salmon Production in the Nanaimo Estuary: II. Meiofauna Available as Food to Juvenile Chum Salmon (*Oncorhynchus keta*)

J. R. SIBERT

Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C. V9R 5K6

SIBERT, J. 1979. Detritus and juvenile salmon production in the Nanaimo Estuary: II. Meiofauna available as food to juvenile chum salmon (*Oncorhynchus keta*). J. Fish. Res. Board Can. 36: 497–503.

The meiofauna of the Nanaimo Estuary are briefly described. Nematodes and harpacticoid copepods were the numerically dominant taxa and reached their maximum population densities in late summer. *Harpacticus uniremis* was very important to the early diet of juvenile chum salmon (*Oncorhynchus keta*) but was one of the least common harpacticoid copepods in the estuary. The productivity of *H. uniremis* was found to be only slightly greater than the calculated food requirement of the chum salmon fry; there was close coupling between prey and predator. The role of detritus and bacterially processed carbon in the feeding of *H. uniremis* are discussed.

Key words: harpacticoid copepods, secondary production, detritus, *Harpacticus uniremis*, turnover ratios, meiofauna

SIBERT, J. 1979. Detritus and juvenile salmon production in the Nanaïmo Estuary: II. Meiofauna available as food to juvenile chum salmon (*Oncorhynchus keta*). J. Fish. Res. Board Can. 36: 497–503.

L'auteur décrit brièvement la méiofaune de l'estuaire de Nanaïmo. Les taxa présents en plus grand nombre sont les nématodes et les copépodes harpacticoïdes dont les populations atteignent leur densité maximale fin été. *Harpacticus uniremis* est un élément très important du régime alimentaire des jeunes saumons keta (*Oncorhynchus keta*) mais un des copépodes harpacticoïdes les moins communs de l'estuaire. On constate que la productivité de *H. uniremis* n'est que légèrement supérieure aux besoins alimentaires calculés des alevins de saumon keta; il y a relation étroite entre proie et prédateur. L'auteur analyse le rôle des détritus et du carbone traité par les bactéries dans l'alimentation de *H. uniremis*.

Received May 29, 1978
Accepted February 1, 1979

Reçu le 29 mai 1978
Accepté le 1 février 1979

STUDIES of meiofauna in relation to predation by fishes have concluded that there is little transfer of material from meiofauna to higher trophic levels (e.g. McIntyre and Murison 1973). In the Nanaimo Estuary, however, juvenile chum salmon (*Oncorhynchus keta*) prey intensively on harpacticoid copepods (Sibert et al. 1977a; Healey 1979). The importance of benthic foods in general and of harpacticoids in particular has been documented for other salmon species and for other estuaries (Kask and Parker 1972; Kaczynski et al. 1973; Mason 1974; Anon. 1974, 1975; Feller and Kaczynski 1975; Sibert and Kask 1978). The purpose of this contribution is to describe the distribution, abundance, and productivity of meiofauna in the Nanaimo Estuary. Harpacticoid copepods, and in particular *Harpacticus uniremis* Kröyer, are considered in detail because of their importance to juvenile salmon.

Methods

A general description of the study area has been presented by Healey (1979). Samples were taken by SCUBA divers at high tide for logistical reasons. Core tubes of areas 6.16 or 8.04 cm^2 were used. Three replicate cores were taken at seven stations in the intertidal zone of the estuary (Fig. 1) at approximately biweekly (22 sample periods) intervals from March 1975 to March 1976. The top 1 cm of sediment in the cores was extruded as soon as the cores were brought to the surface by the divers and preserved in formalin and rose Bengal. The meiofauna were later separated from the sediment by repeated washing and decantation through a 44-μm sieve. The extraction efficiency of this technique depends somewhat on the nature of the sediment. For relatively clean sands, extraction is virtually complete. For finer sediments containing a high percentage of organic debris, efficiency may be as low as 90%. The major difficulty is not in extraction but in recognizing and enumerating animals when there is a large amount of detritus in the extracted sample. All recognizable animals were enumerated to major taxon and the harpacticoid copepods were separated and identified to species.

Results

The regression of harpacticoid copepod counts on core area is given in Fig. 2; counts appear to be a

Printed in Canada (J5260)
Imprimé au Canada (J5260)

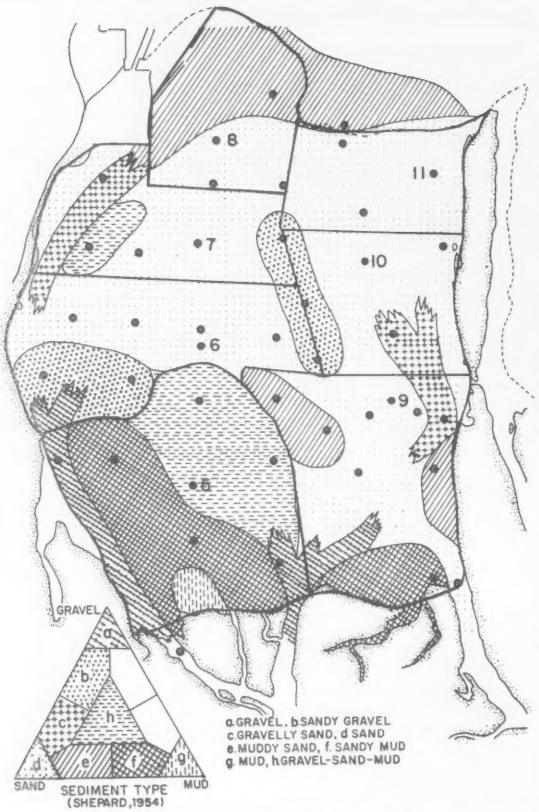


FIG. 1. Nanaimo River Estuary showing locations of meiofauna sampling Stations 5, 6, 7, 8, 9, 10, and 11. Also shown are station locations for sediment analysis (●), distribution of major sediment types, and areas considered to be represented by meiofauna samples. Adapted from Sibert and Reimer (1976).

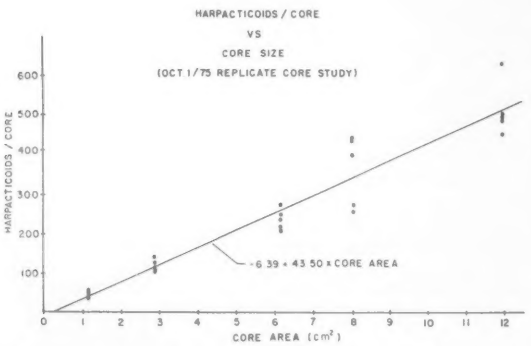


FIG. 2. Regression of number of harpacticoid copepods per core on core area. Straight line is least-squares fit. The hypothesis of zero intercept is acceptable.

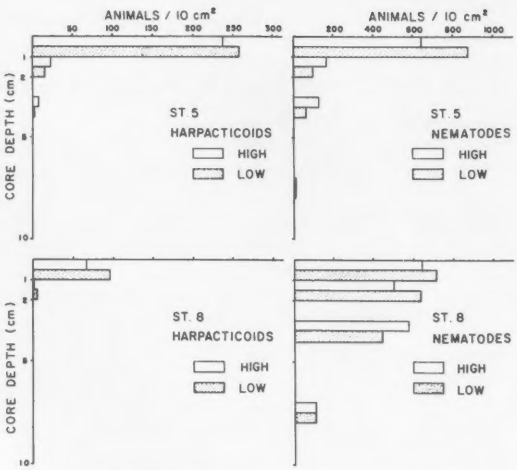


FIG. 3. Vertical distribution of dominant meiofauna taxa, nematodes, and harpacticoid copepods, at two stations in the Nanaimo Estuary at two tidal stages.

simple function of core area. No effect of core size on population density of all taxa was detected by analysis of variance. The vertical distribution of nematodes and harpacticoids in cores at both high and low tides is given in Fig. 3. Most of the animals are found in the surface centimetre of sediment. Although there seem to be differences in meiofauna distribution related to tidal stage, no effect on the number of animals of all taxa occurring in the surface centimetre of sediment could be detected in replicate core samples by analysis of variance. All subsequent discussions pertain to meiofauna found in the surface centimetre of sediment collected by coring at high tide.

Nematodes and harpacticoids were the numerically dominant taxa averaging 508 and 245 individuals/10 cm^2, respectively, over all samples ($n = 413$). Microannelids, amphipods, cumaceans, and ostracods were of secondary importance; other groups were present in small numbers (Table 1). The seasonal changes in the nematode and harpacticoid populations, averaged over all seven stations, are shown in Fig. 4. The nematode population was generally highest and exhibited spring and late summer/fall peaks. The harpacticoid population reached its maximum density in late summer and fall. The maximum in adult population density was preceded by a spring maximum in nauplius density. There were gravid female harpacticoids present in low numbers through the year.

Over 50 different taxa of harpacticoid copepods, including some undescribed species, were identified from the core samples. The most common harpacticoid taxa from the core samples (Table 2) differed greatly from the most common harpacticoid taxa found in chum salmon diets (Healey 1979). *Harpacticus*

TABLE 1. Numbers of individuals per 10 cm² averaged over all 413 core samples.

Nematodes	508.2
Harpacticoids	245.0
Microannelids	67.4
Ostracods	11.6
Amphipods (gammarids)	9.3
Cumaceans	5.1
Ectoproct larvae	2.5
Pelecypods	2.1
Acarina	0.2
Gastropod eggs	0.2
Tanadaceans	0.1
Gastropods	<0.1

uniremis, the single most important harpacticoid species in fry diets, was not a common copepod in core samples when averaged over all stations and season. *Huntemannia jadensis*, an interstitial species, was the only species abundant in cores and also present in fry diets. *Harpacticus uniremis* was common only from mid-March through the end of June (Fig. 5). Its peak abundance was at Station 6 in late May, where it was the numerically dominant species and averaged 66 animals/10 cm². *Harpacticus uniremis* did not occur in samples from Station 5 and the maximum densities at the other stations ranged from 5 to 20 individuals/10 cm². The 90% confidence limits around the mean of the replicate cores were quite large and in some cases the lower limits were negative and so were arbitrarily set to zero. These limits are therefore approximate and should be considered to be qualitative indications of the variability in the data. Precise limits could be calculated but, given the present usage and small sample size (usually 3), are not justified.

Average population density of *H. uniremis* at each station was calculated by dividing the area under the curves in Fig. 5 by the number of days over which the curve was integrated. For example, the area under

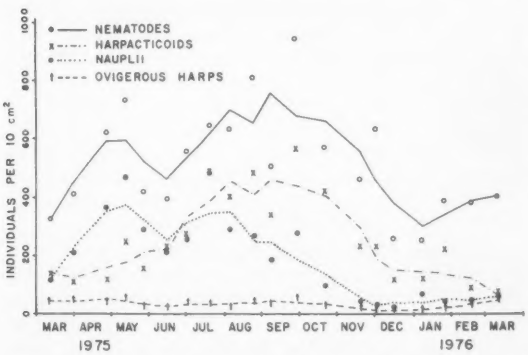


FIG. 4. Seasonal changes in the population density of nematodes and harpacticoids averaged over all stations. Lines are the unweighted 3-point moving average.

TABLE 2. Numbers of harpacticoid copepods per 10 cm² averaged over all core samples (n = 400).

Huntemannia jadensis	50.5
Parastenhelia hornelli	41.1
Family Ectinosomidae	37.6
Schizopera knabeni	35.8
Stenhelia sp. nov.	27.4
Amphiascus undosus	16.5
Copepodites	12.4
Tachidius discipes	9.1
Microarthridion littorale	8.9
Mesochra pygamaea	8.5
Paralaophonte congenera congenera	6.1
Enhydrosoma uniarticulatum	5.6
Nitocra spinipes	1.7
Harpacticus uniremis	1.6
Scottolana canadensis	1.5
Others (35 taxa)	<1.5

the average population density at Station 6 from March 13 to June 16 (96 d) is 2238.7 animal-days/10 cm² and the average density is therefore 23.3 animals/10 cm². For stations where *H. uniremis* was present, average population density ranged between 2.75 and 23.3 animals/10 cm² (Table 3).

The distribution of *H. uniremis* can be interpreted in relation to the characteristics of the sediments (Table 4). *Harpacticus uniremis* was absent from Station 5 where the sediments were poorly sorted sand–gravel–muds. The largest population was observed at Station 6 where the sediments were fine-grained muddy sands. The remaining stations (7, 8, 9, 10, and 11) yielded

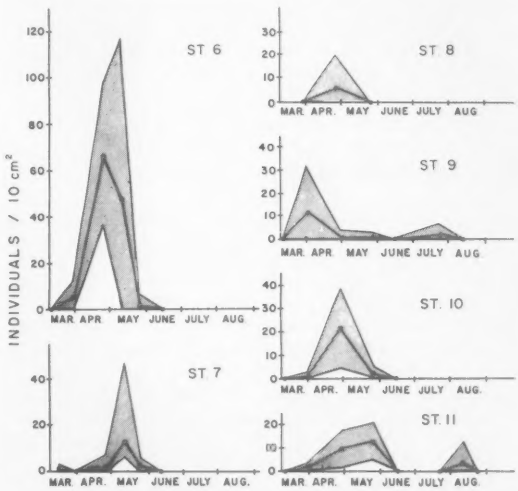


FIG. 5. Seasonal changes in the population density of *Harpacticus uniremis* at Stations 6–11. Closed circles indicate the mean of replicate samples (usually three); shaded areas are the approximate 90% confidence intervals.

TABLE 3. Average population density and productivity of *Harpacticus uniremis* with approximate 90% confidence intervals.

Station	Fraction of flats	Population density (numbers/10 cm²) Lower	Mean	Upper	Weighted density (numbers/10 cm²) Lower	Mean	Upper
5	0.23	0	0	0	0	0	0
6	0.16	17.4	23.3	43.4	2.81	3.77	7.02
7	0.12	0	2.87	10.6	0	0.35	1.27
8	0.11	0	2.75	9.64	0	0.29	1.03
9	0.18	0	3.44	9.89	0	0.63	1.82
10	0.11	2.33	6.85	13.3	0.25	0.73	1.40
11	0.09	1.69	6.09	11.1	0.15	0.55	1.00
Sum	1.00				3.21[a]	6.32[a]	13.55[a]
Average biomass ($mg \cdot m^{-2}$)[b]					71	140	310
Total biomass (kg)[c]					460	940	2000
Productivity ($kg \cdot yr^{-1}$)[d]					4600	9400	20000

[a]Sum of weighted density = average density.
[b]Average biomass = average density × $22.8\ 10^{-3}$ mg·animal^{-1}.
[c]Total biomass = average biomass × 6.5×10^6 m².
[d]Productivity = average biomass × *TR*; *TR* = 10.

intermediate populations and the sediments were uniform well-sorted sands.

The average population density of *H. uniremis* over the whole intertidal flat was calculated by weighting station averages by a factor related to the proportion of the flat represented by each station. An obvious criterion for assigning such weightings is sediment grain size and the weightings become the proportion of the flat consisting of sediments considered to have a uniform composition (Fig. 1). When these weightings were applied to the average population density and to lower and upper confidence limits for each station, the average population density of *H. uniremis* over the entire intertidal flat was between 3.2 and 13.6 with a mean of 6.3 animals/10 cm² (Table 3).

While these data do not permit direct calculations of *H. uniremis* productivity, two indirect estimates can be calculated. One estimate is the product of the average biomass and the turnover ratio (*TR*). The *TR* is defined as the ratio of the production over a period of time to the average biomass during that period, and, for some invertebrates, can be considered to be a constant (Waters 1969; Winberg 1971). The average dry weight of adult and copepodite stages of *H. uniremis* from the Nanaimo Estuary was 2.71 μg·animal^{-1} (Brown and Sibert unpublished data). Using a wet:dry weight ratio of 8.4 (Fulton 1972), the average wet weight of *H. uniremis* was 22.8 μg·animal^{-1}. This factor, applied to the average population density, gives an average wet biomass of between 71 and 310 with a mean of 140 $mg \cdot m^{-2}$ or between 460 and 2000 with a mean of 940 kg for the whole intertidal flat (Table 3). Waters (1969) suggests that the *TR* for species with a single generation per year is 3.5 and that for species with more than one generation, the *TR* will be 2 or 3 times higher. In Alaska, *H. uniremis* produced a single generation per year (Jewett and Feder 1977) but gravid females collected from the Nanaimo Estuary produced as many as 11 broods in laboratory cultures. McIntyre (1964) suggested a *TR* of 10 for meiofauna in general. Gerlach (1971) calculated a *TR* for nematodes of over 25 but suggested that 9 may be more appropriate for other meiofauna. Using a *TR* of 10, the productivity of *H. uniremis* was between 4600 and 20 000 with a mean of 9400 kg/yr (Table 3).

A second estimate of productivity was made from the annual maximum population density (Table 5). The maximum population density was averaged over the 7 stations as in Table 3 and is considered a minimum estimate of productivity. By this method, the total production for the whole intertidal flat ranged between 990 and 5700 with a mean of 2700 kg/yr (wet weight). This estimate is equivalent to that which would have

TABLE 4. Characteristics of surficial sediments at meiofauna stations. At some stations two samples were obtained at different seasons and the range is reported. Percentages are by weight; gravel, >2.0 mm; sand, 2.0–0.0625 mm; mud, <0.0625 mm. Mean and standard deviation (SD) are in ϕ units ($\phi = -\log_2$ mm). Data from Sibert and Reimer (1976).

Station	Percent gravel	Percent sand	Percent mud	Mean	SD
5	12–23	45–42	32–36	2.8	3.0
6	0.8	67	32	3.5	1.8
7	0.1–1.0	90–93	7.0–8.9	2.7	1.6
8	0–1.7	78–87	12–22	3.6	1.4
9	1.5	91	7.2	1.2	1.8
10	11	75	15	1.2	1.8
11	0.4–2.7	96–97	1.3–2.8	1.5	0.9

TABLE 5. Productivity of *Harpacticus uniremis* calculated from maximum population density with approximate 90% confidence limits.

Station	Fraction of flats	Maximum density (numbers/10 cm²)			Weighted maximum (numbers/10 cm²)		
		Lower	Mean	Upper	Lower	Mean	Upper
5	0.23	0	0	0	0	0	0
6	0.16	35	66	117	5.8	11	20
7	0.12	0	12	47	0	1.4	5.6
8	0.11	0	5.6	20	0	0.60	2.1
9	0.18	0	12	32	0	2.1	5.8
10	0.11	4.8	22	39	0.50	2.3	4.1
11	0.09	4.5	13	21	0.41	1.1	1.9
Sum	1.00				6.7[a]	18[a]	38[a]
Productivity ($mg \cdot m^{-2} \cdot yr^{-1}$)[b]					150	420	880
Total productivity ($kg \cdot yr^{-1}$)[c]					990	2700	5700
Productivity + mortality ($kg \cdot yr^{-1}$)[d]					4100	5800	8800
Turnover ratio[e]					8.9	6.2	4.4

[a]The sum of the weighted maximum density = productivity (animals/10 $cm^2 \cdot yr^{-1}$).
[b]Productivity = previous line $\times$ 22.8 $\times$ 10^{-3} $mg \cdot animal^{-1}$.
[c]Total productivity = productivity $\times$ 6.5 $\times$ 10^{-6} m^2.
[d]Productivity + mortality = total productivity + 3100 kg consumed by chum fry.
[e]*TR* = (productivity + mortality) ÷ total biomass (Table 3).

been obtained from the average biomass had a *TR* of 2.9 been applied.

Discussion

Jewett and Feder (1977) reported on a population of *H. uniremis* from Port Valdez, Alaska. The Nanaimo population was similar to the Alaska population with respect to population densities and seasonality with one minor exception. The Alaska population persisted through the summer into September whereas the Nanaimo population declined sharply in June. This discrepancy could be attributed to a variety of factors including differences in growth rate and predation pressure.

Healey (1979) calculated that the juvenile chum salmon required 3100 kg (wet weight) of harpacticoids during their estuarine residence. This requirement is comparable to *H. uniremis* production if a *TR* of about 3.3 is used and is similar to the production estimate by the maximum population density calculation. The 3100 kg consumed by chum fry can be added to the production giving a higher estimate of 5800 kg. Using the average biomass from Table 3, this last estimate is equivalent to a *TR* of 6.2. Pacific herring (*Clupea harengus pallasi*), threespine stickleback (*Gasterosteus aculeatus*), shiner perch (*Cymatogaster aggregata*), and chinook salmon (*Oncorhynchus tshawytscha*) fry are known to feed intensively on harpacticoids on intertidal flats in the Nanaimo Estuary (Sibert and Kask 1978). If these other fish are also exploiting *H. uniremis* populations, then this unaccounted predatory loss could bring the *TR* up to the value of 10 recommended by McIntyre (1964) and Gerlach (1971).

Harpacticus uniremis is found in a variety of habitats. Lang (1948, p. 323) reports it from mud and sand bottoms and from algae. Ito (1971) collected it from algal washings. Jewett and Feder (1977) sampled it from the upper 2–3 cm of sediment. At Nanaimo, *H. uniremis* was collected from the upper 1 cm of sediment and from the layer of water within 10 cm of the sediment surface over the intertidal sand flats and in *Zostera* sp. beds (Sibert et al. 1977b and unpublished data). The distribution of chum fry (Healey 1979) was not congruent to the distribution of *H. uniremis* populations. Is it possible that the fry were exploiting unsampled harpacticoid populations? If that were the case, and assuming the fry were feeding in an area generously estimated to cover 4×10^4 m^2, the average *H. uniremis* population density would have to approach 340 animals/10 cm^2 to produce the 3100 kg of food consumed by the chum fry. This density is far in excess of those observed for *H. uniremis* and approaches the maximum for any single harpacticoid species.

Harpacticus uniremis was a relatively uncommon harpacticoid species in the Nanaimo Estuary. It was rarely present in large numbers and was the dominant harpacticoid species at only one station for a brief period in May. Calculations of its productivity are roughly equivalent to the calculated food demand of the chum fry. The decline in the *H. uniremis* population was coincident with the emigration of chum fry from the study area (Healey 1979). The *H. uniremis* populations partially recovered at some stations after the fry emigration (Stations 9 and 11, Fig. 5). These observations are indicative of close coupling between predator and prey. That the salmon fry should so completely exploit such a marginal food resource is per-

plexing and must be due to the joint behavior of both fish and copepod. *Harpacticus uniremis* adults are reasonably large (~1.5 mm), distinctively marked, and live near the sediment–water interface. Thus they may be the most apparent epibenthic copepods. Unfortunately, very few observations of fry feeding on the epibenthos are available and no relevant field observations of harpacticoid behavior are available.

It is possible to calculate the food resources required to support the *H. uniremis* production. Lasker et al. (1970) report that the interstitial harpacticoid, *Asellopsis intermedia* consists of 32.6% carbon and utilizes about 30% of assimilated carbon for growth. Using these figures, a wet:dry ratio of 8.4, and production estimates using $TR = 10$, the amount of carbon required to support the calculated *H. uniremis* production ranged between 600 and 2600 with a mean of 1200 kg carbon. *Harpacticus uniremis* is capable of assimilating bacterial carbon ~10 times more rapidly than algal carbon. Brown and Sibert (1977) reported assimilation rates of 3.3 and 0.37 $g\,C \cdot kg^{-1}$ dry $wt \cdot h^{-1}$ for bacterial and algal carbon, respectively. At these rates, the *H. uniremis* population could assimilate sufficient algal carbon by feeding continuously for 123 d or could assimilate sufficient bacterial carbon by feeding for 2.7 h/d over the same 123 d. Since the *H. uniremis* populations generally persisted for <123 d, bacterial carbon and, by implication, organic detritus are of great importance to harpacticoid production. This conclusion is further supported by the observation of Naiman and Sibert (1979) that benthic microalgal primary productivity was lowest at Station 6 where *H. uniremis* attained its maximum population density.

The relationship between *H. uniremis* and the rest of the meiofauna can be examined by making rough calculations of the productivity and organic carbon requirements of the total harpacticoid and nematode populations (Fig. 4). The average harpacticoid density for the year was 354 animals/10 cm^2. The productivity of the population was 380 000 kg (wet weight) and the carbon requirement was 49 000 kg ($= 7.5\ g\,C \cdot m^{-2}$) using the same assumptions as for *H. uniremis*. Data on the composition and production of nematodes are harder to find. Assuming an individual dry weight of 2.83 μg, a carbon content of 0.78 μg C (Sikora et al. 1977), $TR = 25$, wet:dry ratio of 4 (Gerlach 1971), and using the annual average nematode population of 508 animals/10 cm^2, the nematode production is 930 000 kg and the carbon requirement is 64 000 kg ($= 33\ g\,C \cdot m^{-2}$). Therefore, on an annual basis, *H. uniremis* contributes little to the total meiofauna production or to the total meiofaunal carbon demand.

Acknowledgments

I thank T. J. Brown, B. A. Kask, and B. Windecker for their participation in all phases of this work. Dr R. Hammond, Zoology Department, University of Melbourne, Australia, and Dr B. Coull, Belle W. Baruch Institute for Marine Biology and Coastal Research, University of South Carolina, kindly provided taxonomic relief. Dr Coull, Dr M. C. Healey, Dr R. J. Naiman, Dr P. A. Breen, and Dr J. C. Mason offered critical reviews of the manuscript.

ANON. 1974. An environmental assessment of Nanaimo port alternatives. Lands Directorate, Can. Dep. Environ., Ottawa, Ont.

1975. Fisheries and food web components of the Fraser River Estuary and an assessment of the impact of proposed expansion of the Vancouver International Airport and other developments on these resources. Fish. Mar. Serv. Can. Dep. Environ., Vancouver, B.C.

BROWN, T. J., AND J. R. SIBERT. 1977. Food of some benthic harpacticoid copepods. J. Fish. Res. Board Can. 34: 1029–1031.

FELLER, R. J., AND V. W. KACZYNSKI. 1975. Size selective predation by juvenile chum salmon (*Oncorhynchus keta*) on epibenthic prey in Puget Sound. J. Fish. Res. Board Can. 32: 1419–1429.

FULTON, J. 1972. Keys and references to the marine copepoda of British Columbia. Fish. Res. Board Can. Tech. Rep. 313: 63 p.

GERLACH, S. A. 1971. On the importance of marine meiofauna for benthos communities. Oecologia (Berlin) 6: 176–190.

HEALEY, M. C. 1979. Detritus and juvenile salmon production in the Nanaimo estuary. I. Production and feeding rates of juvenile chum salmon (*Oncorhynchus keta*). J. Fish. Res. Board Can. 36: 488–496.

ITO, T. 1971. The biology of a harpacticoid copepod, *Harpacticus uniremis* Kroyer. J. Fac. Sci. Hokkaido Univ Ser. 6, Zool. 18: 235–255.

JEWETT, S. C., AND H. M. FEDER. 1977. Biology of the harpacticoid copepod, *Harpacticus uniremis* Kroyer, on the Dayville Flats, Port Valdez, Alaska. Ophelia 16: 111–129.

KACZYNSKI, V. W., R. J. FELLER, AND J. CLAYTON. 1973. Trophic analysis of juvenile pink and chum salmon (*Oncorhynchus gorbuscha* and *O. Keta*) in Puget Sound. J. Fish. Res. Board Can. 30: 1003–1008.

KASK, B. A., AND R. R. PARKER. 1972. Data Report. Estuarine pollution Squamish Estuary, B.C., 1972. Fish. Res. Board Can. MS Rep. 1209: 135 p.

LANG, K. 1948. Monographie der harpacticiden. Otto Koeltz Science Publishers, Koenigstein. (1975 reprint)

LASKER, R., J. B. J. WELLS, AND A. D. MCINTYRE. 1970. Growth reproduction, respiration and carbon utilization of the sand-dwelling harpacticoid copepod, *Asellopsis intermedia*. J. Mar. Biol. Assoc. U.K. 50: 147–160.

MASON, J. C. 1974. Behavioral ecology of chum salmon fry (*Oncorhynchus keta*) in a small estuary. J. Fish. Res. Board Can. 31: 83–92.

MCINTYRE, A. D. 1964. Meiobenthos of sub-littoral muds. J. Mar. Biol. Assoc. U.K. 44: 665–674.

MCINTYRE, A. D., AND D. J. MURISON. 1973. The meiofauna of a flat-fish nursery ground. J. Mar. Biol. Assoc. U.K. 53: 93–118.

NAIMAN, R. J., AND J. R. SIBERT. 1979. Detritus and juvenile salmon production in the Nanaimo estuary. III. Importance of detrital carbon to the estuarine ecosystem. J. Fish. Res. Board Can. 36: 504–520.

SIBERT, J., T. J. BROWN, M. C. HEALEY, B. A. KASK, AND R. J. NAIMAN. 1977a. Detritus-based food webs: exploitation by juvenile chum salmon (*Oncorhynchus keta*). Science 196: 649–650.

SIBERT, J., AND B. A. KASK. 1978. Do fish have diets? In B. G.

Shepherd and R. M. J. Ginetz (rapporteurs) Proceedings of the 1977 Northeast Pacific Chinook and Coho Salmon Workshop. Fish. Mar. Serv. Tech. Rep. 759: 164 p.

SIBERT, J., B. A. KASK, AND T. J. BROWN. 1977b. A diver-operated sled for sampling the epibenthos. Fish. Mar. Serv. Tech. Rep. 738: 19 p.

SIBERT, J., AND P. REIMER. 1976. Textural analysis of the surficial sediments of the Nanaimo River Delta. Fish. Res. Board Can. MS Rep. 1405: 21 p.

SIKORA, J. P., W. B. SIKORA, C. W. ERKENBRECHER, AND B. C. COULL. 1977. Significance of ATP, carbon, and calorific content of meiobenthic nematodes in partitioning benthic biomass. Mar. Biol. 44: 7–14.

WATERS, T. F. 1969. The turnover ratio in production ecology of freshwater invertebrates. Am. Nat. 103: 173–185.

WINBERG, G. G. 1971. Methods for the estimation of production of aquatic animals. Academic Press Inc., New York, N.Y. 176 p.

Detritus and Juvenile Salmon Production in the Nanaimo Estuary: III. Importance of Detrital Carbon to the Estuarine Ecosystem

R. J. NAIMAN[1] AND J. R. SIBERT

Department of Fisheries and Oceans, Resource Services Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6

NAIMAN, R. J., AND J. R. SIBERT. 1979. Detritus and juvenile salmon production in the Nanaimo Estuary: III. Importance of detrital carbon to the estuarine ecosystem. J. Fish. Res. Board Can. 36: 504–520.

Sources of autotrophic and allochthonous organic carbon available to the Nanaimo Estuary delta, British Columbia, were studied from 1974 to 1978. Annually, benthic microalgae produce 4–55 g $C \cdot m^{-2}$, phytoplankton ~7.5 g $C \cdot m^{-2}$, and macroalgae 0.9–7.5 g $C \cdot m^{-2}$. *Zostera marina* (26.8 g $C \cdot m^{-2} \cdot yr^{-1}$) and Carex (~564 g $C \cdot m^{-2} \cdot yr^{-1}$) are productive but enter the food web as detritus. Allochthonous sources are most important with organic matter from the river, especially dissolved organic carbon (~2000 g $C \cdot m^{-2} \cdot yr^{-1}$) and fine particulates (56 g $C \cdot m^{-2} \cdot yr^{-1}$), contributing greatest amounts. The standing crop of organic detritus in the top 5 cm of sediments averages from 58 to 233 g $C \cdot m^{-2}$, depending upon the station. The timing of organic inputs are important, however. Seventy to 93% of total annual river inputs occur during autumn freshets, *Zostera* enters the food web during winter, *Carex* may contribute in early spring, and algae are productive over summer months. Activity and biomass of microbes are high most of the year in surface sediments but in the water column microbes are relatively active only during warmer months. Surface sediment ATP concentrations range seasonally from 3 to 36 $\mu g \cdot g$ sediment^{-1}; and concentrations in the water column range from <0.2 to 1.5 $\mu g \cdot L^{-1}$. Microbial activity, measured with ^{14}C-glucose, ranges seasonally from 4 to 20% uptake per hour for surface sediments and from <1 to nearly 40% $\cdot h^{-1}$ in the water column. These results are compared with those from other studies and coupled to concurrent studies of meiofauna and salmon ecology to show a link between detritus, microbes, harpacticoid copepods, and the food, growth, and production of juvenile chum salmon (*Oncorhynchus keta*).

Key words: detritus, Nanaimo Estuary, primary production, carbon, microbes, salmon, algae, ATP, chlorophyll, *Zostera*

NAIMAN, R. J., AND J. R. SIBERT. 1979. Detritus and juvenile salmon production in the Nanaimo Estuary: III. Importance of detrital carbon to the estuarine ecosystem. J. Fish. Res. Board Can. 36: 504–520.

Nous avons examiné, de 1974 à 1978, les sources de carbone organique autotrophe et allochtone accessible au delta de l'estuaire de Nanaïmo en Colombie-Britannique. Les algues microscopiques benthiques produisent annuellement 4–55 g $C \cdot m^{-2}$, le phytoplancton ~7,5 g $C \cdot m^{-2}$ et les algues macroscopiques 0,9–7,5 g $C \cdot m^{-2}$. *Zostera marina* (26,8 g $C \cdot m^{-2} \cdot an^{-1}$) et *Carex* (~564 g $C \cdot m^{-2} \cdot an^{-1}$) sont productifs mais entrent dans le réseau alimentaire sous forme de détritus. Les sources allochtones les plus importantes sont la matière organique provenant de la rivière, en particulier le carbone organique dissous (~2 000 g $C \cdot m^{-2} \cdot an^{-1}$) et les fines particules (56 g $C \cdot m^{-2} \cdot an^{-1}$) apportant les plus fortes contributions. La biomasse de détritus organiques dans les 5 cm supérieurs des sédiments a une moyenne s'étalant de 58 à 233 g $C \cdot m^{-2}$, selon la station. L'époque à laquelle se produisent les apports de matière organique est toutefois importante. De 70 à 93% des apports fluviaux annuels totaux se produisent durant les crues automnales, *Zostera* entre dans le réseau alimentaire en hiver, *Carex* peut fournir sa contribution début printemps, et enfin les algues sont productives durant les mois d'été. L'activité et la biomasse des microbes sont élevées durant la plus grande partie de l'année dans les sédiments superficiels, alors que dans la colonne d'eau les microbes ne sont relativement actifs que durant les mois chauds. Les teneurs en ATP des sédiments superficiels varient selon la saison de 3 à 36 $\mu g \cdot g$ sédiment^{-1}; et les teneurs dans la colonne d'eau varient de <0,2 à 1,5 $\mu g \cdot L^{-1}$. L'activité microbienne mesurée au ^{14}C-glucose varie selon la saison d'une assimilation de 4 à 20% par heure pour les sédiments de surface et de <1 à presque 40% $\cdot h^{-1}$ dans la colonne d'eau. Nous comparons ces résultats avec ceux d'autres études et les associons à ceux d'études parallèles de l'écologie de la méiofaune et du saumon, pour démontrer l'existence d'un lien entre détritus, microbes, copépodes harpacticoïdes, d'une part, et nourriture, croissance et production de jeunes saumons keta (*Oncorhynchus keta*), d'autre part.

Received May 29, 1978
Accepted January 22, 1979

Reçu le 29 mai 1978
Accepté le 22 janvier 1979

[1]Present address: Woods Hole Oceanographic Institution, Woods Hole, Mass. 02543, USA.

Printed in Canada (J5261)
Imprimé au Canada (J5261)

BETTER strategies for utilization and management of the oceans' resources might be devised if pathways of carbon transfer and how they differ in various marine ecosystems are understood in more detail. In oceans, the classical food web conception has been plant–herbivore–carnivore, but recently Mann (1972) and Pomeroy (1974) have suggested the dominant oceanic food web may be plant–detritus–microbe–consumer. Investigations of salt marshes and estuaries have shown the food web in these ecosystems to be predominantly detritus-based (Teal 1962; Darnell 1967; Nixon and Oviatt 1973; Woodwell et al. 1977). In estuaries with extensive *Spartina* spp. marshes, food webs are believed to be dependent upon production by marsh grasses processed through the decomposer food chain, although Haines (1977) has shown this may not necessarily be the case. In west coast estuaries, which differ both in physical structure and community composition from their eastern counterparts, detailed studies of carbon flow are lacking. Further, no study has definitely established a link between detritus and exploitation by a commercially important fishery, although a number of investigations have documented relationships between organic detritus and benthic organisms (reviewed by Tenore 1977).

In this study we expand on our preliminary report (Sibert et al. 1977) linking juvenile salmon to a detritus-based food web and examine sources of carbon available to the estuarine mudflat ecosystem. Carbon inputs are estimated and, through the studies of Brown and Sibert (1977), Sibert (1979), Healey (1979), and Naiman and Sibert (1978), the detritus-based food chain is related to juvenile salmon production.

Materials and Methods

SAMPLING

The study area is described by Healey (1979). At 2–3-wk intervals from March 1974 to March 1975 water samples were collected at high tide from four stations over the delta (Fig. 1). Benthic samples were collected from the same stations at 2–4-wk intervals from December 1974 to December 1975. Benthic macroalgae standing crop was estimated in April, July, and October 1974. Standing crop and metabolism of macroalgae and seagrasses were measured from June to August 1978.

PHYSICAL AND CHEMICAL VARIABLES

Chlorophyll *a* (corrected for pheopigments), dissolved organic carbon (DOC), inorganic carbon (IOC), and nutrients (PO_4-P, NO_3-N) were determined by methods outlined in Strickland and Parsons (1972). Chlorophyll *a* was converted to organic carbon using organic carbon:chlorophyll *a* ratios of 30 for phytoplankton and 120 for benthic microalgae (however, see Banse 1977). Salinity and temperature profiles were obtained in situ with a Beckman portable salinometer (Model RS 5/3). Adenosine-5′-triphosphate (ATP) was estimated in the water column by the method of Holm-Hansen and Booth (1966), and

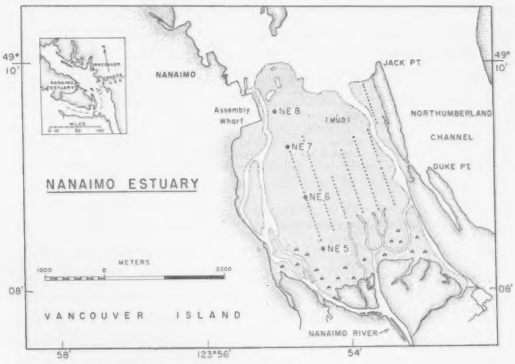


FIG. 1. Nanaimo Estuary mudflat showing position of sampling stations and location of pilings for log rafts.

in sediments by methods of Karl and LaRock (1975). ATP was converted to organic carbon using an organic carbon:ATP ratio of 280. Surface solar radiation (langley (ly) = g cal·cm^{-2}; 1 g cal = 4.19 J) was measured by an Epply Pyranometer at Departure Bay, about 3 km from the estuary (Anon. January 1974–December 1975). Vertical distribution of redox potential (Eh) in the sediments was measured by extruding cores from plexiglass tubes and inserting probes from a Coleman pH meter into the center of the core at various depths. Vertical distribution of carbon in the sediment was studied by fractionating cores at specific depths, grinding the samples to a small size, and analyzing with a Coleman Model 33 Carbon–Hydrogen Analyser.

BIOLOGICAL VARIABLES

Primary production by benthic microalgae was estimated at high tide by injecting 500 μCi (1 Ci = 37 GBq) of $Na_2H^{14}CO_3$ into 3.4 L of water enclosed over the sediment surface by clear and dark plexiglass chambers. After a 4-h midday incubation, chambers were carefully removed by SCUBA divers; six cores were taken and frozen immediately. In the laboratory, the top 1 cm of the core was removed, organics were extracted with nitric acid, and the acid mixture diluted with 0.75 M (mol/L) TRIS. A 1-mL aliquot was counted with a Nuclear-Chicago Liquid Scintillation Spectrometer (Van Raalte et al. 1974).

Phytoplankton production was estimated from samples taken 0.5 m off the bottom at high tide, injected with 5–20 μCi of $Na_2H^{14}CO_3$, and incubated for 5 h at midday in Departure Bay at the depths from which they were taken (Strickland and Parsons 1972).

Standing crop of benthic macroalgae was measured along two or more transects taken from the low tide mark to the marsh by a modified point-centered quarter method (Cottam and Curtis 1956; Foreman 1975). Standing crops of *Zostera marina* and *Ulva fenestrata* were measured again during the period of near-maximum biomass in July 1978. Six 100-m transects were made along the outer margin of the study area, in the eelgrass meadows. In each transect all vegetation, including roots and rhizomes, was removed from 20 randomly selected 0.25 m^2 areas. Samples were dried at 60°C, weighed, and assumed to be 50% carbon.

Metabolism by seagrasses and macroalgae was measured

using sealed, recirculating plexiglass chambers (12.4 L) fitted with oxygen probes and ports for injecting isotopes and taking water samples (Bott et al. 1978). Metabolism was measured by both the diel oxygen curve method (Odum 1956) and, in several cases, by the ^{14}C method (Strickland and Parsons 1972).

Chambers, containing the samples, were placed in large fiberglass tubs connected to a once-through seawater cooling system to keep samples at ambient temperatures. Oxygen concentration was measured with a YSI model 57 O_2-meter, and the results were continuously recorded for 24 h on an Elnik BSC 6-6 strip chart recorder. Chambers were flushed with new estuarine water and the experiments restarted if the oxygen concentration departed from the initial value by more than 4 mg $O_2 \cdot L^{-1}$. As a check on the validity of this experimental method, the ^{14}C-method was also used on several samples. Two hundred and fifty microcuries of $Na_2H^{14}CO_3$ were injected into the chamber, and the samples incubated for 24 h. The results were not significantly different from those of the oxygen method. Therefore, because of the small number of samples using ^{14}C ($n = 6$), only the results for the oxygen method are presented. Bott et al. (1978) give a detailed description of the metabolic chambers and methods of calculating production.

Production by macroalgae can also be estimated by assuming April biomass to be the annual minimum and July biomass the annual maximum; the difference between the two estimates represents net annual production. Biomass data and metabolic rates measured using the sealed plexiglass chambers were also used to estimate net annual production.

Heterotrophic potential at various depths in the sediment was estimated by adding 0.15 μCi of uniformly labeled ^{14}C-glucose (specific activity 310 $\mu Ci \cdot \mu mol^{-1}$) to 0.6 mL of sediment, in 30 mL of estuarine water from the sampling station filtered through a 0.22-μm filter, and incubating for 1 h in the dark at the temperature from which the sample was taken (Sibert and Brown 1975). Glucose added was <0.3 $\mu g\ C \cdot L^{-1}$, low enough to avoid stimulatory effects. After incubation, the sample was treated with 2 mL of concentrated sulfuric acid. To determine the rate of respired $^{14}CO_2$, we introduced 0.5 mL of 50% v/v monoethanolamine through a serum cap into a plastic cup containing a folded filter paper. CO_2 was allowed to diffuse into the monoethanolamine for 24 h. This method recovers 64% of respired $^{14}CO_2$ (Sibert and Brown 1975); respiration rates were corrected accordingly. Heterotrophic potential is reported as rate of total uptake (V_t), in percentage of ^{14}C taken up per hour ($\% \cdot h^{-1}$).

River discharge and organic transport are described in Naiman and Sibert (1978) and are only summarized here. Marsh production was not specifically estimated during this project; an estimate of its production in a nearby area is taken from the literature.

Results and Discussion

Physical Environment and Nutrient Regime

Temperature — Mean water temperatures over the mudflat range from about 2°C in February to 22°C in late August (Table 1). During winter, bottom temperatures are higher (6°C) than surface water temperatures (2–4°C) because of intrusion of saline water from the Strait of Georgia underneath the freshwater lens. Differences among stations at one time are small, usually <3°C.

Salinity — Salinities in the estuary are greatly influenced by wind and freshwater discharge from the river. Station NE5, near the river mouth, has a low salinity for most of the year, but at minimum river discharge during late summer, salinities increase to ~30‰ (Table 1). Salinities at all other stations are usually >20‰, but will decrease to <10‰ when river discharge is high. At outer stations (NE7 and NE8), the estuary is typically two-layered, with seawater intruding onto the mudflat as less saline surface waters flow seaward. Greatest daily oscillations are observed during winter; higher and more constant salinities occur in late summer.

TABLE 1. Summary of the physical environment and nutrient regime on the Nanaimo Estuary mudflats from March 1974 to March 1975.

Station	Temp (°C)			Salinity (‰)		
	n	$\bar{x} \pm$ SD	Range	n	$\bar{x} \pm$ SD	Range
NE 5	25	9.7±6.0	1.8–22.1	27	12.9±9.7	0.2–26.2
NE 6	27	10.3±5.4	2.8–21.8	29	17.4±9.6	1.6–31.1
NE 7	27	10.2±4.8	3.8–19.0	29	20.2±8.6	2.4–30.9
NE 8	29	10.2±5.1	2.1–17.9	27	19.5±9.1	2.1–30.8

Station	NO_3-N(μg-atoms$\cdot L^{-1}$)			PO_4-P(μg-atoms$\cdot L^{-1}$)			DOC(mg C$\cdot L^{-1}$)		
	n	$\bar{x} \pm$ SD	Range	n	$\bar{x} \pm$ SD	Range	n	$\bar{x} \pm$ SD	Range
NE 5	28	11.8±10.4	0.2–26.2	24	1.8±1.5	0.23–3.14	21	1.48±0.50	0.87–2.65
NE 6	29	12.8±10.8	0–27.3	25	1.6±0.8	0.19–2.58	25	1.26±0.42	0.49–2.27
NE 7	29	14.4±11.3	0–28.0	25	1.5±0.9	0.16–2.53	23	1.18±0.40	0.53–1.95
NE 8	29	14.1±10.7	0–27.8	25	1.5±0.9	0.15–2.52	21	1.39±1.00	0.67–2.06

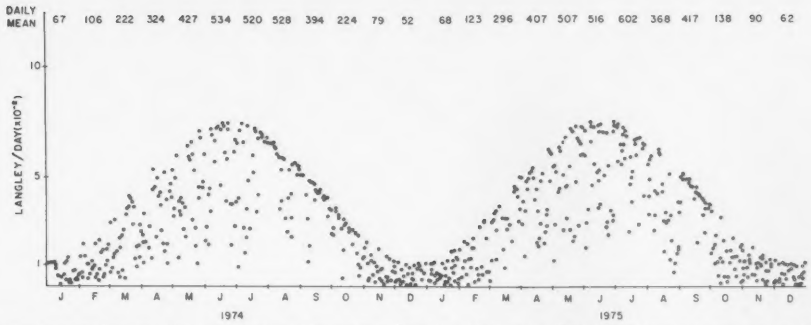


FIG. 2. Daily surface radiation (ly) received at Departure Bay in 1974 and 1975. Numbers across top of figure are mean daily radiation for each month.

Nutrients — NO_3-N and PO_4-P concentrations have the same general seasonal pattern (Table 1), highest in winter and lowest in summer. Nutrient concentrations are most variable during periods of high river discharge and when phytoplankton populations are waxing and waning in spring and autumn.

Redox potential — Water overlying sediments is well oxidized with a mean annual redox potential (Eh) ranging from 290 to 445 mv. However, the redox potential sharply decreases with sediment depth; at the sediment surface it is ~300 mv, declining to ~100–150 mv at a depth of 5 cm. Below 5 cm the redox potential decreases only slightly. A depth of 5 cm for the aerobic layer is assumed for purposes of estimating standing stock of organic carbon available for heterotrophic processing, since the Eh indicates this depth is oxidized for some part of the year. On a mean annual basis, there is no difference in Eh profiles among stations.

Light — Annual distribution of sunlight is variable; during overcast summer days radiation may be limited to typical winter levels (Fig. 2). Lowest levels are in December and January when only 50–60 ly·d^{-1} are received, compared with 600–780 ly·d^{-1} from May through July. In 1974 106 000 ly reached the estuary; the 1975 input was 108 000 ly.

Log storage on the intertidal mudflats affects the light regime by directly shading the sediments and by increasing turbidity through sediment disturbance. Substantial turbidity is also evident during freshets.

BENTHIC MICROALGAE

Species composition — The benthic microalgal community is dominated by diatoms. Surveys conducted in May, June, and September 1975 found 64 species of benthic diatoms at the four stations. Diversity was highest in May and September when 11–26 species were found per station and lowest in late June when <9 species were present at any given station. Few diatoms are found in winter. Only *Navicula gregaria*, *Nitzschia fontifuga*, and *N. frustulum v. perpussila* are common at most times at all stations. Other species become locally abundant at one or two stations for part of the year (*Cocconeis scutellum*, *Cocconeis* spp.). In general, the diatom community appears to be a complex mixture of estuarine, riverine, and marine species.

Chlorophyll — Most chlorophyll *a* is found at the sediment surface but there is a substantial amount in sediments up to 10 cm deep (Fig. 3). Chlorophyll concentrations range from 2.6 to 10.6, and average ~8.4 μg·g sediment^{-1} at the surface, decreasing to ~2 μg·g sediment^{-1} at 10 cm. There is a significant relationship ($r = -0.62$, ANOVA, $P < 0.01$) between mean sea-

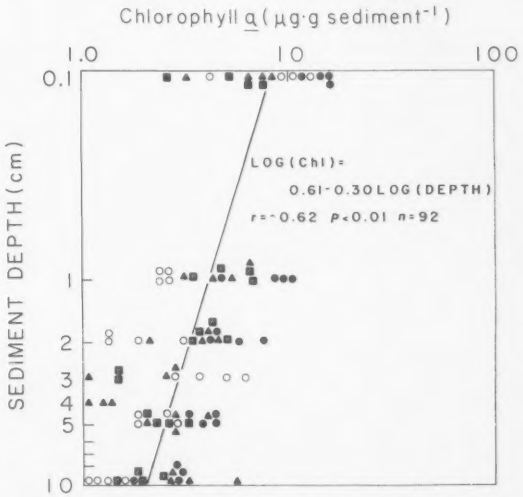


FIG. 3. Mean seasonal chlorophyll *a* concentrations are shown in response to sediment depth. Most chlorophyll is at the surface and concentrations decrease with sediment depth. Symbols are (○) winter, (●) spring, (▲) summer, (■) autumn.

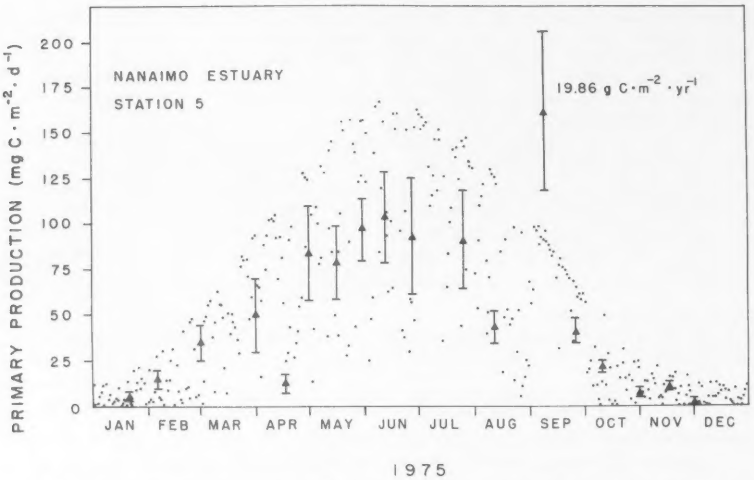


FIG. 4. Daily primary production (●) by benthic microalgae for 1975 at NE5 is predicted from sunlight data and equations in Table 2. Actual values measured ($\bar{x} \pm$ SE) are given as triangles and vertical bars.

sonal chlorophyll concentration and depth. Using seasonal averages we could not detect any significant differences between stations (ANOVA, $P > 0.05$). Within seasons coefficients of variation (CV) among stations range from ~50 to 100% of the mean but are not necessarily greatest at the surface. There are differences among seasons, but because of the spatial heterogeneity of concentrations at any one time, a significant relation could not be shown.

The vertical distribution of viable chlorophyll in marine sediments is well documented (see Pamatmat 1968). Most chlorophyll is usually at the sediment surface but significant amounts, associated with diatoms and blue-green algae, may be found to depths of 20 cm or more. Certain groups of diatoms are able to migrate a few millimetres in wet, soft sediments to maintain maximum photosynthetic potential (Pomeroy 1959); occurrences at greater depths probably result from sedimentation or mixing of surface sediments with deeper layers. Some diatoms can exist at these depths for a considerable period without light and without becoming hetereotrophic (Munro and Brock 1968), yet show significant photosynthetic potential when resuspended (Grontved 1962; Pamatmat 1968; Riznyk and Phinney 1972). This may be important to diatoms on the Nanaimo Estuary sandflat where log-handling activities frequently scour much of the area.

The standing crop of surface chlorophyll on the sandflat is <100 mg chlorophyll $a \cdot m^{-2}$, which is low when compared with concentrations in many other areas (Odum et al. 1958; Pamatmat 1968; Estrada et al. 1974; Matheke and Horner 1974). It is comparable to that found on sandy beaches (Steele and Baird 1968), or where the substrate is highly shaded or frequently disturbed. In the Nanaimo Estuary both of the latter conditions are associated with logging activities and may be primary factors in the low amount of chlorophyll associated with surface sediments.

The Nanaimo Estuary delta has relatively large concentrations of nonfunctional chlorophyll, however. Ratios of optical density at 665 μm before and after acidification normally range between 1.15 and 1.25 for surface algae. The ratio increased to the 1.25–1.60 range on only a few occasions in spring and summer. This contrasts with the findings of Steele and Baird (1968) in which the ratio ranges between 1.4 and 1.8 at all depths in a sandy beach.

Microalgal production — Primary production by benthic microalgae is significantly correlated ($r =$ 0.68–0.88, ANOVA, $P < 0.01$) with surface sunlight during the course of a year (Table 2). All stations show an increasing power curve relationship between primary production and solar radiation. There is no significant difference (ANOVA, $P > 0.05$) in slope of lines between stations but there is a significant difference ($P < 0.01$) in intercepts. For this reason each station is analyzed separately. Because of considerable variation in sunlight at this study location, daily production was estimated from solar radiation–primary production equations in Table 2. Empirical and estimated values of production agree closely (Fig. 4, only one station shown). Most production takes place when >300 ly $\cdot d^{-1}$ reach the water surface. Little production occurs between October and March when sunlight levels are greatly reduced by short, cloudy days and low water transparency caused by high river discharge.

All stations have low annual production rates (Table

TABLE 2. Statistical parameters used to estimate primary production (mg $C \cdot m^{-2} \cdot d^{-1}$) by benthic microalgae and phytoplankton from total surface radiation (1 langley = 1 g $cal \cdot cm^{-2}$; 1 cal = 4.19 J). $^{**}P < 0.01$.

Station	Equation	Correlation coefficient (r)	Sample size (n)	Mean water depth (m)
	Phytoplankton			
NE 5	1.98 log (ly)–3.95	0.75**	20	0.24
NE 6	2.43 log (ly)–5.02	0.80**	22	0.85
NE 7	2.18 log (ly)–4.24	0.85**	22	1.46
NE 8	1.47 log (ly)–2.63	0.59**	22	2.07
	Benthic microalgae			
NE 5	1.35 log (ly)–1.66	0.88**	18	
NE 6	1.00 log (ly)–1.41	0.70**	13	
NE 7	1.50 log (ly)–2.06	0.68**	13	
NE 8	2.60 log (ly)–4.57	0.83**	18	

3). Station NE6, in an area highly disturbed by log rafts, produces only 4.22 g $C \cdot m^{-2} \cdot yr^{-1}$, while NE8, near an eelgrass meadow and less disturbed by outside activities, produces 55.46 g $C \cdot m^{-2} \cdot yr^{-1}$. NE5 and NE7 each produces about 20 g $C \cdot m^{-2} \cdot yr^{-1}$. Average annual production is 25.05 g $C \cdot m^{-2} \cdot yr^{-1}$.

Annual net production by benthic microalgae on the intertidal flats is somewhat lower than that found for benthic algae elsewhere (Table 4). Only NE8 has a rate comparable to other data from a similar latitude. Station NE6 has the same production rate as a sandy beach, where waves make the sediment unstable. Stations NE5 and NE7, adjacent to areas of logging activity, have rates only 0.3–0.5 as great as those found in other studies. The low production rates at all stations should probably be expected considering the relatively low standing stock of surface chlorophyll over most of the mudflat.

Efficiencies — The sparce benthic algae community is not very efficient in its use of sunlight for photosynthesis (Table 5). On an annual basis microalgae utilize only 0.02–0.10% of surface radiation available for photosynthesis. The actual efficiency should be slightly higher because surface radiation is measured, and not subsurface sunlight.

Pomeroy (1959) found efficiences in Georgia varying during the year from 1–3% at low light levels to 0.1% at high light levels, and an annual value of 0.3%. Pamatmat (1968) estimated an annual efficiency of 0.4% for benthic algae in Washington. Given that net annual production and photosynthetically available light incident on the water surface are being used to estimate efficiencies, it is reasonable to assume that the efficiency estimate of 0.10% at NE8 may be comparable to those found by Pomeroy and Pamatmat. However, even with these assumptions, the photosynthetic efficiencies for Stations NE5, NE6, and NE7 are only 0.2–0.3 of those found in Georgia and Washington.

The sparse development of the microalgal component is also reflected by the ratio of annual carbon production to mean annual standing crop (i.e. turnover rate) of microalgae in surface sediments (Table 5). At NE8 the mean standing crop of chlorophyll is replaced ~8.9 times each year; this decreases to 4.3 at NE7, 2.4 at NE5, and is only 0.5 at NE6. These low turnover rates are predominantly a function of production rates at each station, since all stations have similar concentrations of chlorophyll (<0.1 g $\cdot m^2$) in surface sediments which are much less than the estimated maximum chlorophyll concentration (0.4–0.8 g $\cdot m^{-2}$) needed if light is to be maximally utilized by algae to produce new organic carbon (Steeman Nielsen 1962).

PHYTOPLANKTON

Chlorophyll — Chlorophyll *a* concentrations in water over the delta steadily increase from May to October before returning to wintertime levels (Fig. 5). Spikes of chlorophyll *a* (14–19 $\mu g \cdot L^{-1}$) occur in spring during short-lived, or patchy, phytoplankton blooms. Typical summer values are between 1 and 7 $\mu g \cdot L^{-1}$; winter values are $< \mu g \cdot L^{-1}$. There are no significant differences among stations but NE5 and NE6 tend to have lower summer concentrations.

Phytoplankton production — Net autotrophic production in the water column is significantly correlated ($r = 0.59$–0.85, ANOVA, $P < 0.01$) with surface solar radiation (Table 2). Annual production by phytoplankton is estimated for each station by using mean water depth and equations relating production to solar radiation for each station. Production is relatively high from May to September and low for the remainder of the year (Fig. 6). Annual production ranges from 1.22

TABLE 3. Primary production of benthic microalgae on the Nanaimo Estuary in 1975 (g $C \cdot m^{-2} \cdot mo^{-1}$).

Station	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Total
NE 5	0.22	0.44	1.56	2.25	3.09	3.10	3.85	2.06	2.23	0.55	0.31	0.20	19.86
NE 6	0.08	0.13	0.36	0.47	0.60	0.59	0.72	0.44	0.48	0.17	0.11	0.08	4.22
NE 7	0.18	0.40	1.55	2.33	3.31	3.32	4.19	2.12	2.29	0.51	0.26	0.16	20.61
NE 8	0.09	0.39	2.87	5.74	9.99	10.87	14.37	5.28	5.17	0.49	0.14	0.06	55.46
Mean	0.14	0.34	1.59	2.70	4.25	4.47	5.78	2.48	2.54	0.43	0.21	0.13	25.05

TABLE 4. Annual production by benthic marine microalgae.

Location	Annual production ($g\,C \cdot m^{-2} \cdot yr^{-1}$)	Reference
Scotland	4–9	Steel & Baird (1968)
British Columbia	4–55	This study
Scotland	31	Leach (1970)
Delaware	40[a]	Gallagher & Daiber (1974)
Massachusetts	53[b]	Van Raalte et al. (1976)
Denmark	58[a]	Grøntved (1960)
Oregon	0–163[a]	Riznyk & Phinney (1972)
Washington	53–74[a,c]	Pamatmat (1968)
Georgia	180	Pomeroy (1959)

[a]Assuming net production to be 50% of gross production.
[b]Assuming dry weight to be 50% carbon.
[c]Assuming 1 g carbon = 2 L O_2.

$g\,C \cdot m^{-2}$ at NE5 to 13.2 $g\,C \cdot m^{-2}$ at NE7, with an average annual production of 7.48 $g\,C \cdot m^{-2}$ over the mudflat (Table 6).

Chlorophyll *a* concentrations and primary production in the water column are comparable in magnitude and seasonality to surface water in deeper areas nearby. Stations several kilometres from the mudflat produce 7–13 $g\,C \cdot m^{-2} \cdot yr^{-1}$ in the upper 1–2 m of water where the euphotic zone is ~20 m (Brown et al. 1976). These production values are similar to those from other areas in the Strait of Georgia. Parsons et al. (1970) estimate phytoplankton production to be 120 $g\,C \cdot m^{-2} \cdot yr^{-1}$ for the euphotic zone of open waters in the Strait of Georgia while Stockner et al. (1977) give estimates ranging from 118 to 515 $g\,C \cdot m^{-2} \cdot yr^{-1}$ for various parts of Howe Sound, British Columbia. There is little doubt, considering the tidal water exchange with the

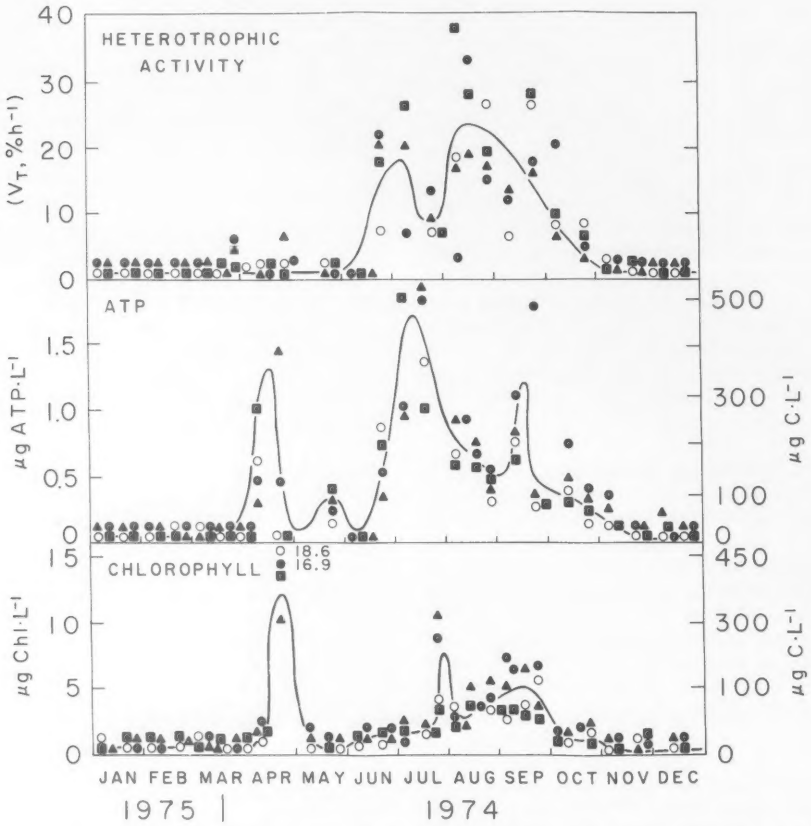


FIG. 5. Heterotrophic activity (V_t), ATP, and chlorophyll *a* of water collected 0.5 m above the sediment are shown for an annual period. Symbols are (○) NE5, (■) NE6, (▲) NE7, (●) NE8.

TABLE 5. Annual efficiencies of benthic microalgae are given in relation to annual surface radiation and mean standing crop of chlorophyll *a*.

	Station			
	NE8	NE6	NE7	NE8
Light efficiency (%)	0.04	0.02	0.04	0.10
Chlorophyll (turnover ratio)	2.36	0.50	4.29	8.92

These calculations assume
1 langley = 1 g cal·cm^{-2}; 1 g cal = 4.19 J
1 g C = 10 kcal
a carbon:chlorophyll *a* ratio of 120
only 47% of total radiation is photosynthetically active.

Strait of Georgia, that waters over the estuary have the same characteristics as offshore surface water.

Efficiencies — The phytoplankton turnover rate tends to be slow during most of the year (Fig. 6). From April to October the turnover rate for all stations normally remains between 4 and 10% ·d^{-1}. However, during peak production in spring when biomass is low, the turnover rate may go as high as 25% ·d^{-1}. From October to March, when both phytoplankton production and biomass are low, the turnover rate remains near zero. Chlorophyll *a* concentrations are maximally 5–10 μg·L^{-1} in the top 5 m of offshore water and have a turnover time similar to water over the mudflats.

Light efficiency for the phytoplankton component remains near zero throughout the year. Most photosynthetically available light reaches bottom sediments and is not utilized by phytoplankton. During peak production periods the efficiency is only 0.01–0.10%.

MACROPHYTES

Composition and zonation — Three major plant zones characterize the intertidal mudflat. Zone I, nearest the *Carex* marsh, covers 25 ha and contains mostly *Fucus* and *Salicornia*. Zone II (438 ha), between Zone I and the outer margin of the mudflat, contains *Ulva* and *Enteromorpha*. Zone III (184 ha), along the outer margin of the mudflat in deeper water, contains *Zostera* and *Ulva*.

A total of 37 benthic macroalgae species are found on the mudflat from April to October, but only six of those species are present during the entire period (*Enteromorpha* sp., *E. intestinalis*, *E. linza*, *Fucus distichus*, *Porphyra* sp., and *Ulva fenestrata*). More species are present in summer and autumn than during spring.

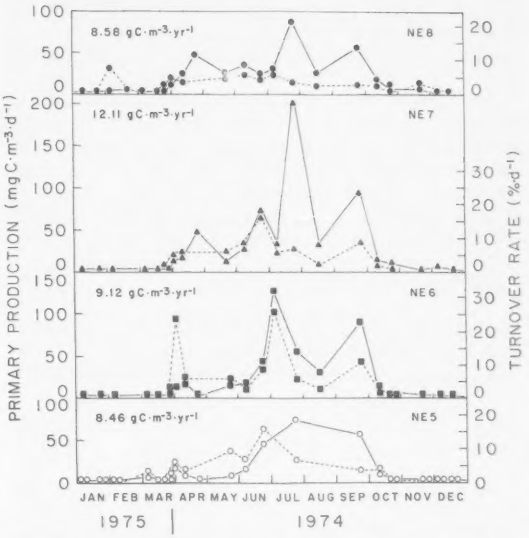


FIG. 6. Phytoplankton primary production (—) and turnover rates (- - -) in water over the Nanaimo mudflat are shown for an annual period.

Macroalgae standing crop and production — Maximum biomass occurs in July and the minimum in April (1% of July) (Table 7). Two problems, however, are encountered with Zone I data. First, the samples included individuals of *Salicornica virginica*. Although they constitute a significant fraction of the biomass in Zone I, these plants are excluded from the calculations because they are perennials. Second, no samples were taken in Zone I in April and July. The combined Zone II and III biomass for July is 5.13 times that for October and the April estimate is 0.01 times that for July. Zone I biomass estimates for July and April are calculated by applying these factors to the October data.

TABLE 6. Monthly production by phytoplankton (mg C·m^2·mo^{-1}).

Station	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
NE 5	5.0	15.0	77.3	88.0	158.3	235.9	242.7	217.2	123.7	43.0	6.6	3.0	1215.9
NE 6	12.7	50.2	331.4	400.8	801.3	1297.5	1336.6	1142.4	574.6	160.9	18.1	6.8	6133.4
NE 7	40.2	135.9	783.7	915.3	1727.0	2671.9	2751.1	2413.2	1300.7	410.3	54.9	23.1	13227.5
NE 8	85.1	185.3	699.7	750.4	1190.3	1604.3	1646.9	1546.1	1011.8	452.7	103.7	58.2	9334.5
Mean	35.8	96.6	473.0	538.6	969.2	1452.4	1494.3	1329.7	752.7	266.7	45.8	22.8	7477.9

TABLE 7. Biomass of intertidal benthic macroalgae in the Nanaimo Estuary in 1974. Figures are in g C·m^{-2}.

Month	Zone I	Zone II	Zone III
April	0.15[a]	0.01	0.01
July	12.75[a]	0.54	0.34
October	2.49	0.11	0.07
July–April	12.60	0.53	0.33

[a]Estimated from Zone I and II data.

TABLE 8. Standing crop (g C·m^{-2}) of seagrasses and macroalgae in Zone III of the Nanaimo Estuary mudflat, July 1978.

Transect	*Zostera* (vegetative) $\bar{x}\pm$SE	*Zostera* (flowering) $\bar{x}\pm$SE	Macroalgae $\bar{x}\pm$SE
I Jack Point	53.1±24.1	2.3±1.7	2.3±1.3
II Gravel Bar	65.9± 9.0	0.6±0.3	45.4±7.2
III Off Gravel Bar	0.0± 0.0	0.0±0.0	0.6±0.5
IV NE 8	11.2± 7.8	0.9±0.9	1.1±0.8
V Assembly Wharf	21.1± 8.6	0.2±0.2	2.9±1.6
VI Wharf Light	65.8±14.3	10.6±4.6	9.4±3.8
$\bar{x}$	36.2±10.6	2.4±1.3	10.3±2.5
SE	11.8	1.7	7.1

Biomass of macroalgae on the mudflats is small when compared with other estuaries. Pomeroy and Stockner (1976) report standing crops of 12–322 g C·m^{-2} in a British Columbia estuary; Nixon and Oviatt (1973) found peak densities of 130–300 g C·m^{-2} in a New England salt marsh; and Conover (1958) found a range ~100–200 g C·m^{-2} near Woods Hole, Massachusetts. On the Nanaimo Estuary mudflat, however, total biomass is nearly always <2.5 g C·m^{-2} (Table 7). Reasons for the low biomass are not known but it may be due to the unstable substrate and tidal currents.

An estimate of net production can be obtained by assuming July biomass for each zone to be the annual maximum and the April biomass to be the minimum. The difference between the two estimates is a minimum net production estimate. When each zone is prorated by area, the average net production is calculated to be ~0.94 g C·m^{-2}·yr^{-1}.

In July 1978 the standing crop of macroalgae was determined again in Zone III, the area of maximum biomass, and the metabolism of *U. fenestrata* measured (Table 8 and 9). The 1978 standing crop was several times higher than in July 1975. These data, when combined with measurements of metabolic activity and the following assumptions, give an independent estimate of annual production: (1) the growing season is from April to the end of October, (2) the standing crop is constant, (3) production rates are constant throughout the growing season, and (4) *Entermorpha* and *Ulva* have the same metabolic rates. These assumptions will cause an overestimate of annual production, possibly by a factor of 2–3, because biomass increases from near zero in April, to a maximum in August, and decreases to near zero by the end of November. Also, the metabolic rates measured are probably near the maximum, since the experiments were conducted during midsummer in relatively warm water and with clear skies. The data, however, are sufficient to give an estimate for comparison with other sources of carbon available to the ecosystem.

Annual production by macroalgae, using this method, is calculated to be 7.5 g C·m^{-2}. This estimate is higher than that obtained from standing crop data alone, but supports the contention that macroalgae are not especially important producers of carbon in the system. If these production values are realistic, the turnover rate would range from 0.8–3.0, indicating that the standing crop replaces itself ~1–3 times each year.

Eelgrass standing crop and production — The standing crop of *Zostera marina* exceeds that of macroalgae (*U. fenestrata* and *Enteromorpha*) in Zone III (Table

TABLE 9. Summary of metabolic activity experiments for *Zostera marina* and *Ulva fenestrata*.

Species	Temp range (°C)	Sample size (n)	Metabolic (mg C·g dry wt^{-1}·d^{-1}) parameter ($\bar{x}$ ± SE) NP[a]	R_n[b]	R_{24}[c]	GP[d]	NDM[e]
Zostera marina (vegetative turions)	14.0–21.2	18	8.27±1.03	2.08±0.17	5.84±0.48	14.11±1.47	6.19±0.95
Z. marina (flowering turions)	18.0–21.2	3	6.65±1.24	2.44±0.45	6.14±1.14	12.79±2.26	4.22±0.98
Ulva fenestrata	18.5–20.5	6	29.25±4.88	9.81±1.13	21.03±2.80	50.29±5.13	19.44±5.58

[a]Net production (NP) = rate of reduction of inorganic carbon, minus the rate of oxidation by respiration during the photoperiod.
[b]Night time respiration (R_n).
[c]Diel respiration (R_{24}).
[d]Gross production (GP) = $NP + R_{24}$.
[e]Net daily metabolism (NDM) = $NP - R_n$.

8). *Zostera* is reported to have been much more abundant in the Nanaimo Estuary prior to about 1950. At that time, the estuary was essentially undisturbed and eelgrass was reported growing over two thirds of the area. Since 1950, eelgrass meadows have been drastically reduced, possibly due to increased siltation caused by logging activity in the watershed and by log storage over the intertidal delta (Bell and Kallman 1976). At present, clumps of eelgrass occur over 17.9% of the study area at elevations <2 m above mean lowest low tide.

The distribution and density of eelgrass in Zone III is not uniform (Table 8). Locally dense concentrations occur near a gravel bar near the middle of the mudflat, at Jack Point, and near the wharf light. Flowering turions of *Z. marina* occur in patches throughout the eelgrass meadows but are relatively dense only near the wharf light. Compared to other locations the biomass of *Zostera* is not large. Izembek Lagoon, Alaska, has a maximum *Zostera* standing crop of ~650 g C·m^{-2} in summer (McRoy 1974), compared to 36.2 g C·m^{-2} on the Nanaimo mudflat (Table 8).

The metabolic activity of vegetative *Z. marina* is slightly greater than that of the flowering forms averaging ~6.19 mg C·g dry wt^{-1}·d^{-1} (Table 9). These rates are only about one third of *Ulva*. The metabolic rates for eelgrasses reported here are at the low end of the range reported by McRoy (1974) for *Zostera* in Alaska. Depending upon the light intensity, McRoy found net uptake rates ranging from 5 to 93 mg C·g dry wt^{-1}·d^{-1}. Since an oxygen method was used in these experiments the actual metabolic rate would probably be underestimated due to lacunal storage of photosynthetically formed oxygen. However, in the few (n = 6) experiments where both the ^{14}C and oxygen methods were used, the ^{14}C method confirmed that metabolic activity was low when compared to McRoy's results.

Metabolic data, when combined with measurements of standing crop and the assumptions used for estimating annual production by macroalgae, give a slight overestimate of the yearly carbon input by eelgrasses because standing crop and metabolic rates are not constant. Annual production by vegetative *Z. marina*, in the area where it occurs, is estimated to be 147.7 g C·m^{-2}. This area is only 17.9% of the delta; therefore, the annual contribution to the total mudflat is 26.4 g C·m^{-2}. Annual production by flowering *Z. marina*, because of its relative scarcity, is 2.1 g C·m^{-2}. It also occurs over 17.9% of the delta; therefore, its annual input is 0.4 g C·m^{-2}.

Carbon exchange with the marsh — Production by the *Carex* marsh in the upper portion of the delta, and movement of carbon from the marsh were not investigated. Production values, however, can be applied from other nearby locations. Levings and Moody (1976) estimate an annual production of 661 g C·m^{-2} on the Squamish River marsh and give a value of 467 g C·m^{-2} for the Fraser River marsh in British Columbia. An average of these two values (564 g C·m^{-2}·yr^{-1}) is used for marsh production in the Nanaimo Estuary. Some studies of marsh ecosystems in the eastern United States have shown that 23–45% of marsh production may be exported in particulate form (Teal 1962; Nixon and Oviatt 1973). Other studies, however, failed to show any net export of carbon from marshes to surrounding waters (Heinle and Flemer 1976; Woodwell et al. 1977; Haines 1977). The Nanaimo Estuary marsh is poorly flooded and no marsh detritus was observed on the mudflat. Tentatively, we are assuming no appreciable flux of carbon between the marsh and the mudflat.

Primary Production Summary

Relative production of carbon among primary producers occurs in this order: *Zostera* — benthic microalgae — phytoplankton — benthic macroalgae. Possible limiting factors are light, nutrients, and substrate disturbance. Light is of obvious importance for all plant groups in a cloudy climate. Nutrients become important in spring and summer when they virtually disappear in dissolved form from the water column. Substrate disturbance may be as important as light and nutrients in limiting autotrophic production since sediment disturbance and scouring from log rafts and tug boats adversely affects eelgrass meadows, macroalgae, and benthic microalgae.

Microbial Studies

ATP — Assay of ATP associated with organic detritus offers a convenient means of assessing the standing stock of the microbial community and assists in explaining the cycling and potential availability of carbon in sediments. Surface sediments have higher concentrations of ATP than deeper sediments (Fig. 7). ATP concentration at the surface ranges seasonally from 3 to 36 with a mean of 17.1 μg·g sediment^{-1}, and decreases to 1.5 μg·g sediment^{-1} at 25-cm depth. There is a highly significant relationship between mean seasonal ATP concentration and sediment depth (r = −0.70, ANOVA, $P < 0.01$). Within a season, coefficients of variation for a station range from 27 to 75% of the mean with little regard to depth. There are no significant differences in the vertical profile among stations, except that NE5 approaches zero beyond 15 cm depth.

Highest levels of water column ATP are observed during summer months (0.8 to > 1.5 μg·L^{-1}) and lowest levels (<0.2 μg·L^{-1}) in winter (Fig. 5). Peaks of short duration may occur in spring and are usually associated with phytoplankton blooms.

Surface sediment concentrations of ATP are slightly higher on the Nanaimo mudflats than those reported for other locations. Christian et al. (1975) report values of 1.2–9.8 μg·g sediment^{-1} in a Georgia salt marsh;

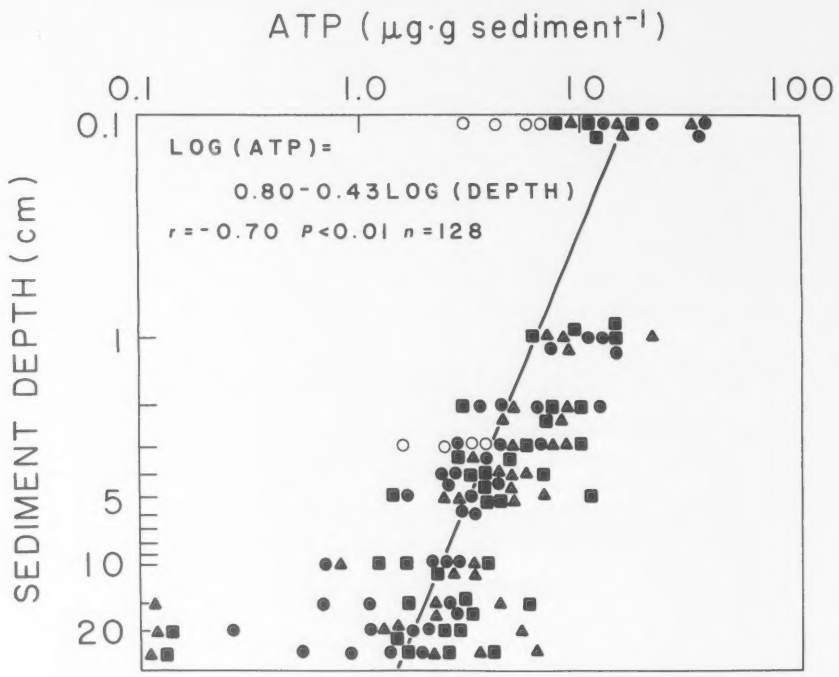


FIG. 7. Mean seasonal ATP content of sediments is plotted as a function of sediment depth. ATP concentrations decrease with sediment depth. Symbols are (○) winter, (●) spring, (▲) summer, (■) autumn.

Lee et al. (1971) measured ATP in temperate lake sediments ranging from 1.5–9.5 $\mu g \cdot g$ sediment^{-1}; and tundra sediments from Alaska have ATP concentrations of 1.1–7.5 $\mu g \cdot g$ sediment^{-1} (Campbell et al. 1973). Values observed in a North Carolina estuary also fall into the above ranges (Ferguson and Murdoch 1975). Sikora et al. (1977) report that the ATP content of marine nematodes is 3.76 ng ATP·nematode^{-1}. Sibert (1979) reports the average population density of nematodes to be 5.08×10^3 nematodes·m^{-2} in the upper 1 cm of sediment. This population density is equivalent to 0.26 μg ATP·g^{-1} of sediment or ~1.5% of the total sediment ATP.

Reasons for the higher ATP concentrations on the Nanaimo Estuary mudflats are unclear, but three considerations may be important. First, the large input of concentrated DOC from the Nanaimo River may be a prime carbon source for estuarine microbes (Naiman and Sibert 1978). Second, scouring associated with log booming activities may increase sediment turnover, thus deepening the redox discontinuity layer and mixing organic carbon in the near-surface sediments. Third, grazing by meiofauna may keep microbes in an active growth phase.

Microbial activity — Microorganisms associated with sediments are probably the primary component in oxidation and cycling of organic detritus. A large research effort has been directed at measuring metabolic rates of sediment microbes, with oxygen uptake used as an indication of activity (reviewed by Pamatmat 1977). However, because that method gives no indication of potential microbial production, we used isotopic methods to measure the potential of sediment microorganisms to assimilate organic detritus (Christian and Hall 1977). This method does not measure actual production but, rather, gives a comparative indication of microbial conversion of detrital carbon to a form potentially available to consumers.

All four stations show decreasing mean seasonal heterotrophic activity (V_t) as sediment depth increases. At Stations NE5, NE6, and NE8 this relationship is significant ($r = -0.82$ to -0.88; ANOVA, $P < 0.01$). At Station NE7 the correlation is not significant ($r = -0.33$, ANOVA, $P < 0.05$) because microbial activity increases just below the sediment surface to ~10 cm and then begins a gradual decline. There are no significant differences in activity levels between stations (ANOVA, $P > 0.05$). When all mean seasonal data are combined there is a significant correlation with sediment depth ($r = -0.68$, ANOVA, $P < 0.01$, $n = 72$) (Fig. 8). The standard error around each data

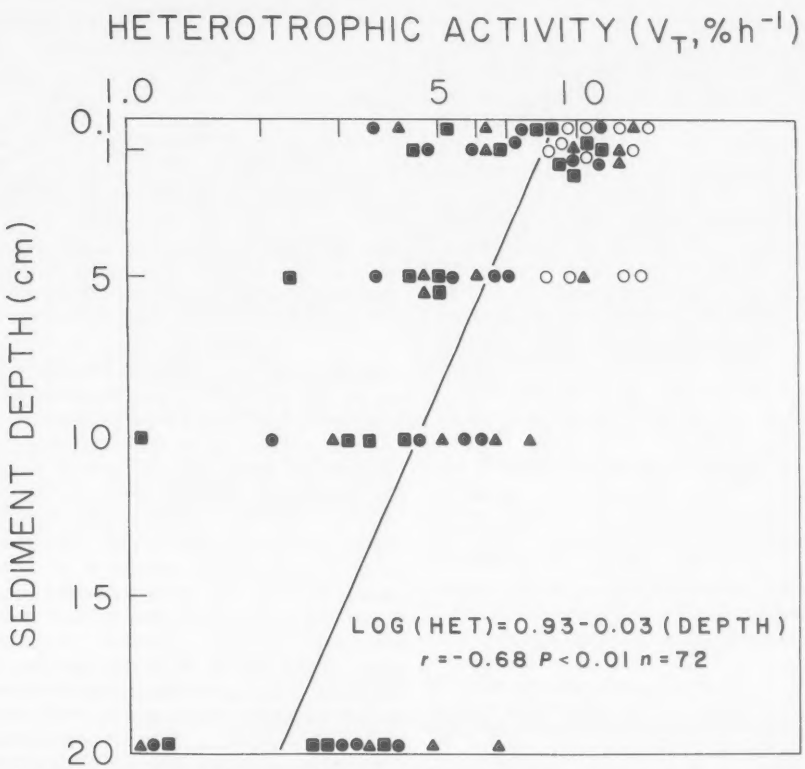


FIG. 8. Mean seasonal heterotrophic activity (V_t) is shown in response to sediment depth. Activity of microbes is greatest at the surface and decreases with depth. Symbols are (○) winter, (●) spring, (▲) summer, (■) autumn.

point is ~0.75–2.00% · h^{-1}; coefficients of variation range from 20 to 60% of the seasonal mean, being skewed toward lower values. Mean seasonal surface activity is 9.4% · h^{-1}, decreasing to 2.1% · h^{-1} at 20 cm.

Carbon normally available to the aerobic community is in the surface sediment. The depth weighted average of microbial activity in the top 5-cm layer is plotted for each station in Fig. 9 and ranges from about 3 to 17% · h^{-1}, depending upon the time of year. All stations have the same general pattern. There is an activity peak from January to March (>10% · h^{-1}), a decrease in April and May (~5% · h^{-1}), another increase over the summer (>10% · h^{-1}, except at NE7), and a decrease in autumn (~3–8% · h^{-1}).

Microbial metabolism in the water column is highest from early June through October (Fig. 5). During these months there is considerable variability associated with V_t as it ranges from 5% · h^{-1} to nearly 40% · h^{-1}. The average during the June–October period is about 20% · h^{-1}; from November to May nearly all rates are <2% · h^{-1}. Station NE5 tends to be low in summer and Station NE6 high, but there are no significant differences among stations.

ATP and heterotrophic activity in the water column over the mudflat also reflect characteristics of offshore surface water. ATP concentrations in surface water at deep-water stations off the mudflat range up to 3.3 μg · L^{-1}, and heterotrophic activity rates typically approach 30% · h^{-1} in summer (Brown et al. 1976).

Few comparisons are available for these data. Hall et al. (1972) studied heterotrophic uptake by sediment microbes in Marion Lake, British Columbia, finding gross uptake rates highest in summer and low levels for the remainder of the year. Sibert and Brown (1975) estimate that heterotrophic conversion of organic matter in industrial wastes easily replace the effects of depressed primary production on the food web in a fjord on Vancouver Island. Both studies show the importance of microbial conversion of organic detritus to microbial biomass, and its ultimate potential to consumers.

DETRITUS

Standing crop — The importance of organic detritus in aquatic ecosystems has received considerable atten-

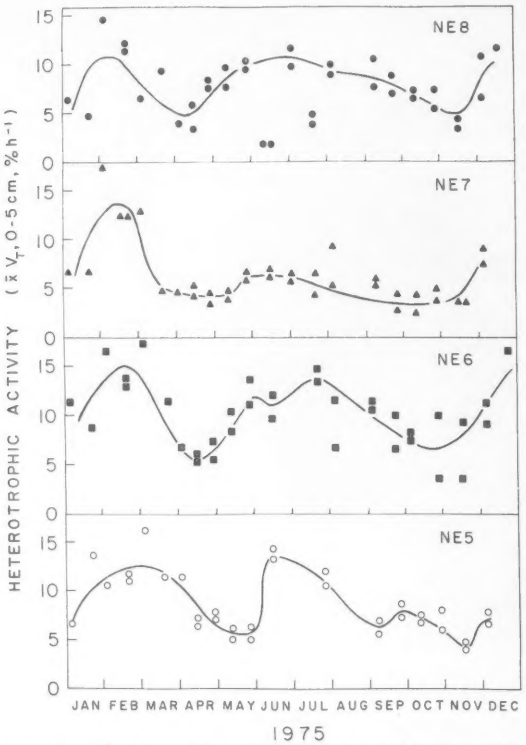


FIG. 9. The depth weighted average of total heterotrophic activity in the upper 5 cm of sediment is given for each station. All stations have maximum activity in winter and summer. Lines are drawn by eye.

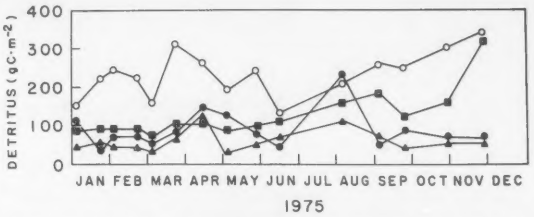


FIG. 10. Total organic carbon in the upper 5 cm of sediment is shown for each station during the study period. Note that detritus increases in the fall at NE5 and NE6. Symbols are (○) NE5, (■) NE6, (▲) NE7, (●) NE8.

tion (Darnell 1967; Melchiorri-Santolini and Hopton 1972); however, most investigators have concentrated on physiological or physiochemical characterization of organic detritus and its associated microorganisms without measuring standing stock of organic detritus available to the food web.

On the Nanaimo Estuary mudflat the standing stock of organic detritus varies from ~50 to >300 g C·m^{-2}, depending upon the station and time of year (Fig. 10). Station NE5 consistently has the largest amount of organic carbon in the upper 5-cm sediment layer with an annual mean of 232.8 g C·m^{-2} (SE = 14.2) and the highest percentage of carbon ($\bar{x}$ = 2.75%, SE = 0.17). Stations NE6, NE7, and NE8 have less detritus, as well as a lesser percentage of carbon, in the top 5-cm layer. Their mean annual values are clustered between 58.1 and 127.2 g C·m^{-2}, and between 0.69 and 1.60% carbon. Stations NE5 and NE6 show an increase in the detrital standing crop during autumn coinciding with maximum river transport of organic matter (Fig. 10).

These values are lower than the 296–384 g C·m^{-2} found by Ferguson and Murdoch (1975) for the Newport River Estuary in North Carolina but comparable to the 100 g C·m^{-2} of macrodetritus found by Nixon and Oviatt (1973) in a New England salt marsh. However, as pointed out by Johnson (1974), conventional methods of representing the amount of organic matter are misleading. Relative to the weight or volume of mineral particles, organic matter appears to be an insignificant component, but still represents a large pool of carbon potentially available to the food web. Johnson shows that most particles encountered by benthic organisms are potential food items of a diverse nature and, taken together, represent an enormous surface area for the growth of microorganisms. Through the biological action of microorganisms and larger animals it is conditioned into a quality food source (Darnell 1964; Welsh 1975) and can be assimilated into higher trophic levels (Adams and Angelovic 1970; Brown and Sibert 1977). The diversity in size and nature of potential food particles is paralleled by a diverse array of strategies for separating organic from inorganic components of the sediment.

River export — Transport of organic carbon from the Nanaimo River onto the mudflats exceeds all other sources (Naiman and Sibert 1978). Most of the annual carbon input occurs during autumn freshets. From October to December the intertidal mudflat receives 70% of the annual dissolved organic carbon (DOC; <0.5 μm) river export, 73% of the annual fine particulate organic carbon (FPOC; >0.5 μm, <1 mm) export, and 93% of the annual large particulate organic carbon (LPOC; >1 mm) export. DOC contributes by far the greatest amount of carbon each year, nearly 2000 g C·m^{-2}. FPOC contributes 56 g C·m^{-2}·yr^{-1}, while LPOC contributes only 0.7 g C·m^{-2}·yr^{-1}.

How much of this fluvial carbon is retained and used by the intertidal estuarine system is not known, but available evidence suggests the amount may be considerable. Rivers carry negatively charged organic particles that are precipitated by positively charged metallic ions of salt water (Darnell 1967; Morris et al. 1978), and investigations have shown rapid precipitation of terrestrially formed organic matter upon contact with seawater (Sieburth and Jensen 1968; Gardner

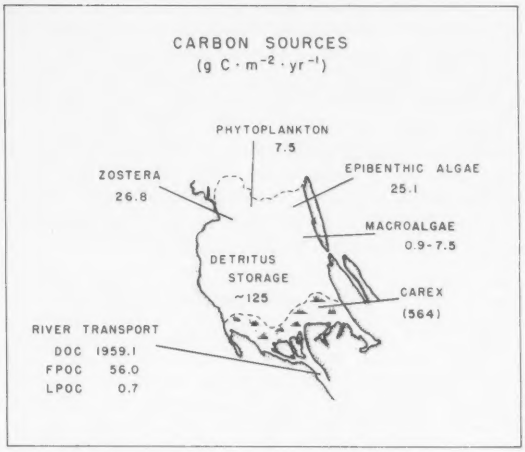


FIG. 11. Sources of carbon available to the Nanaimo Estuary mudflats are shown, and the estimated magnitude of each given in g C · m^{-2}.

and Menzel 1974; Sholkowitz 1976; Morris et al. 1978). Station NE5 nearest the river source has the largest standing crop of detritus and NE5 and NE6 show an increase in the standing crop of detritus during the autumn freshet period (Fig. 10). Correspondingly, the microbial community, as measured by sediment surface ATP and uptake of ^{14}C-glucose, is very active during most of the year at all stations.

Oceanic exchange — The tides at Nanaimo have a daily range averaging ~3 m, causing a large percentage of the water over the mudflat to be exchanged with the Strait of Georgia. This represents a considerably movement of carbon but, because of complex current patterns, it has so far proved impossible to measure net carbon flux.

In terms of volume, annual oceanic exchange is several orders of magnitude greater than river discharge. However, concentrations of organic matter in river water are much higher than in the ocean. DOC averages 6.44 mg C · L^{-1} in the river but only 1.32 mg C · L^{-1} in both the Strait of Georgia and in the water over the mudflat (Naiman and Sibert 1978). FPOC averages ~250 μg · L^{-1} in the river but only 100–125 μg · L^{-1} in the ocean. The ocean has the potential to contribute a larger amount of carbon but the river contribution is more concentrated.

TROPHIC CHARACTERISTICS

Major sources of carbon available to consumer species in the Nanaimo Estuary intertidal area are shown in Fig. 11. The largest contribution is by DOC from the Nanaimo River. Much of this DOC may move through the intertidal area into the Strait of Georgia or it may be of a highly refractile nature, but if only 10% is utilized it would still be the largest contribution. River transport of FPOC is twice the estimated *Zostera* production and exceeds the combined production of macroalgae, epibenthic microalgae, and phytoplankton. The marsh has the potential to supply large amounts of carbon but is poorly flooded by high tides and may not contribute much to the intertidal area. Oceanic exchange, a major component of this system, is missing in Fig. 11. For the present we are assuming no appreciable exchange as found by Woodwell et al. (1977) for Flax Pond. There may actually be a net export from the mudflat to the surrounding area since the delta drops off into deep water very quickly but this hypothesis remains to be tested.

The magnitude of these sources can be compared to the annual meiofaunal carbon requirements calculated by Sibert (1979). Harpacticoids and nematodes, the dominant meiofauna, annually consume ~7.5 and 33 g C · m^{-2}, respectively, or a total of 40 g C · m^{-2}. These estimates are conservatively low because they do not include consumption by other meiofauna or macrofauna and are roughly equivalent to the sum of the annual production by microalgae and eelgrass. There is no corresponding estimate for microbial production but average bacterial biomass, estimated from ATP concentration, is 34 g C · m^{-2} and production must be at least an order of magnitude higher. Thus there would appear to be sufficient heterotrophic production to support the estimated meiofaunal carbon demand. The estimate of 34 g C · m^{-2} for bacterial biomass in the upper centimetre is very high when compared to the average standing crop of detritus (125 g C · m^{-2}) in the upper 5 cm of sediment. Other workers have reported that microbial carbon makes up only ~1% of the total sediment carbon (Christian et al. 1975; Sikora et al. 1977). We offer no explanation of this apparent discrepancy except to note that it is consistent with estimated meiofaunal carbon requirements and that conventional methods of estimating bacterial biomass are notoriously low (Watson et al. 1977).

The trophic structure of the Nanaimo Estuary delta is quite different from that found by Teal (1962) and Nixon and Oviatt (1973) for estuaries in the eastern United States that largely depend upon inputs of detritus from marsh grasses. Woodwell et al. (1977), however, observed the Flax Pond salt marsh to be a carbon sink, trapping carbon from surrounding waters. In Georgia and New England primary production by epibenthic microalgae or macroalgae is an important ecosystem component; at Nanaimo algal primary production is a relatively minor component. In nearby False Bay, Washington, Pamatmat (1968) found algal primary production to be the most important source of carbon to the intertidal mudflat; however, there is no appreciable input of freshwater to False Bay. In west coast estuaries, where marshes are smaller and less sheltered than east coast counterparts, and where river

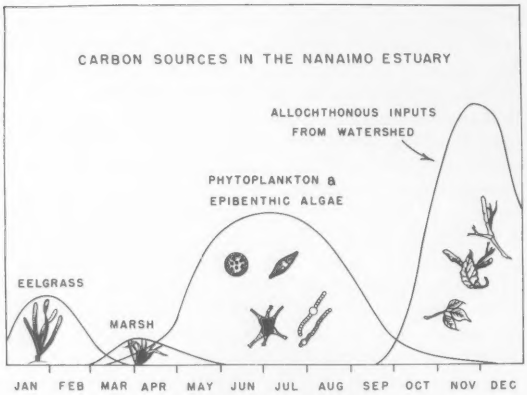

CARBON SOURCES IN THE NANAIMO ESTUARY
ALLOCHTHONOUS INPUTS FROM WATERSHED
PHYTOPLANKTON & EPIBENTHIC ALGAE
EELGRASS
MARSH
JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC

FIG. 12. Diagramatic representation of the timing of organic carbon inputs to the Nanaimo Estuary mudflat from each major carbon source.

discharge is important, the dominant source of carbon may be from terrestrial sources. Seki et al. (1969) have estimated that, in the Strait of Georgia, annual input of carbon from rivers is equal to the total yearly phytoplankton production.

On an annual basis the Nanaimo Estuary is dominated by river inputs but the timing of this and other carbon inputs during the year is such that they complement each other (Fig. 12). Most river inputs occur from October to January and are of relatively minor importance for the remainder of the year. *Zostera* grows over the summer but is mainly available to consumers from December through February when it decomposes. Total decomposition, however, may take years to accomplish (Harrison and Mann 1975). Marsh grasses grow during the summer but, if they are exported to the mudflat, it only occurs on high tides for a few weeks in spring, after the winter dieback. Epibenthic algae and phytoplankton are productive during the longer days of spring and summer. It is evident that each carbon source becomes important to consumer species by its timing in availability to the food web, and not just through the magnitude of its input.

The results of our study support the concept of Mann (1972) and Pomeroy (1974) that the oceans' food web is predominately detrital. Pomeroy presents a paradigm for the oceans' food web consisting of plants–detritus–microbes–consumers. This contrasts with the classical concept of algal production–herbivores–larger consumers, with microbes thought to be a dead end as decomposers and not utilized by consumer species. Our results show that the Nanaimo Estuary mudflat is a detritus-based ecosystem receiving large amounts of carbon from the Nanaimo River. Primary production by *Zostera*, microalgae, and macroalgae are somewhat lower than that in other areas, and are not especially important sources of carbon on an annual basis. However, the timing and availability of each carbon source is important to the system. A large and active microbial community is associated with the detritus, and microbial production appears to greatly exceed in situ primary production. Microbes are consumed by meiofauna (Brown and Sibert 1977; Sibert 1979) of which the harpacticoid copepod, *Harpacticus uniremis*, is a major food item for juvenile chum salmon during their first critical weeks of sea life (Healey 1979). Thus, a direct link has been established between detritus, microbes, and a commercially important fishery.

Acknowledgments

We thank T. J. Brown, K. V. Stephens, and B. Windecker for their excellent technical assistance in the field and laboratory and G. Blair for preparing the illustrations. This work was supported by a National Research Council (Canada) Post Doctoral Fellowship to R. J. Naiman and by funds provided through the Pacific Biological Station. Their financial assistance is gratefully acknowledged. Dr J. G. Stockner and M. Amspoker identified the diatoms. Drs M. C. Healey, P. A. Breen, K. H. Mann, C. D. McAllister, and R. J. Feller critically reviewed drafts of the manuscript. Our appreciation and thanks are extended to all who have helped.

ADAMS, S. M., AND J. W. ANGELOVIC. 1970. Assimilation of detritus and its associated bacteria by three species of estuarine animals. Chesapeak Sci. 11: 249–254.

ANON. 1974–1975. Monthly radiation summary. Fish. Environ. Can. Atmospheric Environ. Serv. January 1974 to December 1975.

BANSE, K. 1977. Determining the carbon-to-chlorophyll ratio of natural phytoplankton. Mar. Biol. 41: 199–212.

BELL, L. M., AND R. J. KALLMAN. 1976. The Nanaimo River estuary: Status of environmental knowledge to 1976. Spec. Estuary Ser. No. 5. Environ. Can. Pac. Environ. Inst. West Vancouver, B.C.

BOTT, T. L., J. T. BROCK, C. E. CUSHING, S. V. GREGORY, D. KING, AND R. C. PETERSON. 1978. A comparison of methods for measuring primary productivity and community respiration in streams. Hydrobiologia 60: 3–12.

BROWN, T. J., AND J. R. SIBERT. 1977. Food of some benthic harpacticoid copepods. J. Fish. Res. Board Can. 34: 1028–1031.

BROWN, T. J., J. SIBERT, AND K. STEPHENS. 1976. Data pertaining to the lower trophic levels at Nanaimo River estuary and adjacent waters from March 1974 to March 1975. Fish. Mar. Serv. Data Rec. 20: 146 p.

CAMPBELL, W. B., R. W. HARRIS, AND R. E. BENOIT. 1973. Response of Alaskan tundra microflora to a crude oil spill. Occas. Publ. North Life 1: 53–62.

CHRISTIAN, R. R., K. BANCROFT, AND W. J. WIEBE. 1975. Distribution of microbial adenosine triphosphate in salt marsh sediments at Sapelo Island, Georgia. Soil Sci. 119: 89–97.

CHRISTIAN, R. R., AND J. R. HALL. 1977. Experimental trends in sediment microbial heterotrophy: Radiosotopic techniques and analysis, p. 67–88. *In* B. C. Coull [ed.] Ecology of marine benthos. Univ. South Carolina Press, S.C. 476 p.

CONOVER, J. T. 1958. Seasonal growth of benthic marine plants as related to environmental factors in an estuary. Publ. Inst. Mar. Sci. 5: 97–147.

COTTAM, G., AND J. T. CURTIS. 1956. The use of distance measurements in phytosociological sampling. Ecology 37: 451–460.

DARNELL, R. M. 1964. Organic detritus in relation to secondary production in aquatic communities. Verh. Internat. Verein. Limnol. 15: 462–470.

1967. Organic detritus in relation to the estuarine ecosystem, p. 376–382. *In* G. Lauff [ed.] Estuaries. AAAS. Washington. 757 p.

ESTRADA, M., I. VALIELA, AND J. M. TEAL. 1974. Concentration and distribution of chlorophyll in fertilized plots in a Massachusetts salt marsh. J. Exp. Mar. Biol. Ecol. 14: 47–56.

FERGUSON, R. L., AND M. B. MURDOCH. 1975. Microbial ATP and organic carbon in sediments of the Newport River estuary, North Carolina p. 229–250. *In* L. E. Cronin [ed.] Estuarine research. Vol. I Academic Press Inc., New York, N.Y. 738 p.

FOREMAN, R. E. 1975. Seasonal aspects of macrophyte distribution and standing crop on the Nanaimo River estuary mudflats. Univ. British Columbia, Dep. Botany, Benthic Ecology Research Program Rep. 75–3: 41 p.

GALLAGHER, J. L., AND F. C. DAIBER. 1974. Primary production of edaphic algal communities in a Delaware salt marsh. Limnol. Oceanogr. 19: 390–395.

GARDNER, W. S., AND D. W. MENZEL. 1974. Phenolic aldehydes as indicators of terrestrially derived organic matter in the sea. Geochim. Cosmochim. Acta 38: 813–822.

GRØNTVED, J. 1960. On the productivity of microbenthos and phytoplankton in some Danish fjords. Medd. Dan. Fisk. Hanund. 3: 55–92.

1962. Preliminary report on the productivity of macrobenthos and phytoplankton in the Danish Wadden Sea. Medd. Dan. Fisk. Hanund. 3: 347–378.

HAINES, E. B. 1977. The origins of detritus in Georgia salt marsh estuaries. Oikos 29: 254–260.

HALL, K. J., P. M. KLEIBER, AND I. YESAKI. 1972. Heterotrophic uptake of organic solutes by microorganisms in the sediment. Mem. Ist. Ital. Idrobiol. 29 (Suppl.): 441–471.

HARRISON, P. G., AND K. H. MANN. 1975. Detritus formation from eelgrass (*Zostera marina* L.): The relative effects of fragmentation, leaching, and decay. Limnol. Oceanogr. 20: 924–934.

HEALEY, M. 1979. Detritus and juvenile salmon production in the Nanaimo Estuary. I. Production and feeding of juvenile chum salmon. J. Fish Res. Board Can. 36: 488–496.

HEINLE, D. R., AND D. A. FLEMER. 1976. Flows of materials between poorly flooded tidal marshes and an estuary. Mar. Biol. 35: 359–373.

HOLM-HANSEN, O., AND C. R. BOOTH. 1966. The measurement of ATP in the ocean and its ecological significance. Limnol. Oceanogr. 11: 510–519.

JOHNSON, R. G. 1974. Particulate matter at the sediment-water interface in coastal environments. J. Mar. Res. 32: 313–330.

KARL, D. M., AND P. A. LAROCK. 1975. Adenosine triphosphate measurements in soil and marine sediments. J. Fish. Res. Board Can. 32: 599–607.

LEACH, J. H. 1970. Epibenthic algal production in an intertidal mudflat. Limnol. Oceanogr. 15: 514–521.

LEE, C. C., R. F. HARRIS, J. D. H. WILLIAMS, J. K. SYERS, AND D. E. ARMSTRONG. 1971. Adenosine triphosphate in lake sediments: II. Origin and significance. Soil Sci. Soc. Am. Proc. 35: 86–91.

LEVINGS, C. D., AND A. I. MOODY. 1976. Studies of intertidal vascular plants, especially sedge (*Carex lyngbyei*), on the disrupted Squamish River delta, British Columbia. Fish Mar. Serv. Tech. Rep. 606: 57 p.

MANN, K. H. 1972. Introductory remarks. Mem. Ist. Ital. Idrobiol. 29 (Suppl.): 13–16.

MATHEKE, G. E. M., AND R. HORNER. 1974. Primary productivity of the benthic microalgae in the Chukchi Sea near Barrow, Alaska. J. Fish. Res. Board Can. 31: 1779–1786.

MCROY, C. P. 1974. Seagrass productivity: carbon uptake experiments in eelgrass, *Zostera marina*. Aquaculture 4: 131–137.

MELCHIORRI-SANTOLINI, U., AND J. W. HOPTON [ed.]. 1972. Detritus and its role in aquatic ecosystems. Mem. Ist. Ital. Idrobiol. 29 (Suppl.): 1–540.

MORRIS, A. W., R. F. C. MANTOURA, A. J. BALE, AND R. J. M. HOWLAND. 1978. Very low salinity regions of estuaries: Important sites for chemical and biological reactions. Nature 274: 678–680.

MUNRO, A. L. S., AND T. D. BROCK. 1968. Distinction between bacterial and algal utilization of soluble substances in the sea. J. Gen. Microbiol. 51: 35–42.

NAIMAN, R. J., AND J. R. SIBERT. 1978. Transport of nutrients and carbon from the Nanaimo River to its estuary. Limnol. Oceanogr. 23: 1183–1193.

NIXON, S. W., AND C. A. OVIATT. 1973. Ecology of a New England salt marsh. Ecol. Monogr. 43: 463–498.

ODUM, H. T. 1956. Primary production in flowing waters. Limnol. Oceanogr. 2: 102–117.

ODUM, H. T., W. MCCONNELL, AND W. ABBOTT. 1958. The chlorophyll *a* of communities. Publ. Inst. Mar. Sci. 5: 65–96.

PAMATMAT, M. M. 1968. Ecology and metabolism of a benthic community on an intertidal sandflat. Int. Rev. Gesamten Hydrobiol. 53: 211–298.

1977. Benthic community metabolism: A review and assessment of present status and outlook, p. 89–111. *In* B. C. Coull [ed.] Ecology of marine benthos. Univ. South Carolina Press, S.C. 476 p.

PARSONS, T. R., R. J. LEBRASSEUR, AND W. E. BARRACLOUGH. 1970. Levels of production in the pelagic environment of the Strait of Georgia, British Columbia: A review. J. Fish. Res. Board Can. 27: 1251–1264.

POMEROY, L. R. 1959. Algal productivity in salt marshes of Georgia. Limnol. Oceanogr. 4: 386–397.

1974. The ocean's food web, a changing paradigm. BioScience 24: 499–504.

POMEROY, W. M. AND J. G. STOCKNER. 1976. Effects of environmental disturbance on the distribution and primary production of benthic algae on a British Columbia estuary. J. Fish. Res. Board Can. 33: 1176–1187.

RIZNYK, R. Z., AND H. K. PHINNEY. 1972. Manometric assessment of interstitial microalgae production in two estuarine sediments. Oecologia 10: 193–203.

SEKI, H., K. V. STEPHENS, AND T. R. PARSONS. 1969. The contribution of allochthonous bacteria and organic materials from a small river to a semi-enclosed sea. Arch. Hydrobiol. 66: 37–47.

SHOLKOWITZ, E. R. 1976. Flocculation of dissolved organic and inorganic matter during mixing of river water and sea water. Geochim. Cosmochim. Acta 40: 831–845.

SIBERT, J. R. 1979. Detritus and juvenile salmon production in the Nanaimo Estuary. II. Meiofauna available as food to juvenile chum salmon. J. Fish. Res. Board Can. 36: 497–503.

SIBERT, J., AND T. J. BROWN. 1975. Characteristics and

potential significance of heterotrophic activity in a polluted fjord estuary. J. Exp. Mar. Biol. Ecol. 19: 97–104.

SIBERT, J., T. J. BROWN, M. C. HEALEY, B. A. KASK, AND R. J. NAIMAN. 1977. Detritus-based food webs: Exploitation by juvenile chum salmon (*Oncorhynchus keta*). Science 196: 649–650.

SIEBURTH, J. MCN., AND A. JENSEN. 1968. Studies on algal substances in the sea. I. Gelbstoff (humic material) in terrestrial and marine waters. J. Exp. Mar. Biol. Ecol. 2: 174–189.

SIKORA, J. P., W. B. SIKORA, C. W. ERKENBRECHER, AND B. C. COULL. 1977. Significance of ATP, carbon, and caloric content of meiobenthic nematodes in partitioning benthic biomass. Mar. Biol. 44: 7–14.

STEELE, J. H., AND I. E. BAIRD. 1968. Production ecology of a sandy beach. Limnol. Oceanogr. 13: 14–25.

STEEMAN NIELSEN, E. 1962. On the maximum quantity of plankton chlorophyll per surface unit of a lake or the sea. Int. Rev. Gesamten. Hydrobiol. 47: 333–338.

STOCKNER, J. G., D. D. CLIFF, AND D. B. BUCHANAN. 1977. Phytoplankton production and distribution in Howe Sound, British Columbia: a coastal marine embayment-fjord under stress. J. Fish. Res. Board Can. 34: 907–917.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1972. A practical handbook of seawater analysis. Bull. Fish. Res. Board Can. 167: 310 p.

TEAL, J. M. 1962. Energy flow in the salt marsh ecosystem of Georgia. Ecology 43: 614–624.

TENORE, K. R. 1977. Food chain pathways in detrital feeding benthic communities: A review with new observations on sediment resuspension and detrital recycling, p. 37–53. *In* B. C. Coull [ed.], Ecology of marine benthos. Univ. South Carolina Press, S.C. 467 p.

VAN RAALTE, C. D., W. C. STEWART, I. VALIELA, AND E. J. CARPENTER. 1974. A ^{14}C technique for measuring algal productivity in salt marsh muds. Bot. Mar. 17: 186–188.

VAN RAALTE, C. D., I. VALIELA, AND J. M. TEAL. 1976. Production of epibenthic salt marsh algae: light and nutrient limitation. Limnol. Oceanogr. 21: 862–872.

WATSON, S. W., T. J. NOVITSKY, H. L. QUINBY, AND F. W. VALOIS. 1977. Determination of bacterial number and biomass in the marine environment. Appl. Environ. Microbiol. 33: 940–946.

WELSH, B. L. 1975. The role of grass shrimp, *Palaemonetes pugio*, in a tidal marsh ecosystem. Ecology 56: 513–530.

WOODWELL, G. M., D. E. WHITNEY, C. A. S. HALL, AND R. A. HOUGHTON. 1977. The Flax Pond ecosystem study: Exchanges of carbon in water between a salt marsh and Long Island Sound. Limnol. Oceanogr. 22: 833–838.

Net Entanglements of Baleen Whales in the Inshore Fishery of Newfoundland

J. S. PERKINS

150 Chestnut St., Weston, MA 92193, USA

AND P. C. BEAMISH[1]

Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, N.S. B2Y 4A2

PERKINS, J. S., AND P. C. BEAMISH. 1979. Net entanglements of baleen whales in the inshore fishery of Newfoundland. J. Fish. Res. Board Can. 36: 521–528.

This paper documents net entrapments of baleen whales in the inshore waters of Newfoundland and Labrador. The species involved are principally minke whales (*Balaenoptera acutorostrata*) and humpback whales (*Megaptera novaeangliae*). Finback whales (*Balaenoptera physalus*) are also caught in nets but far less frequently. The timing of these entrapments corresponds with the spawning season for capelin (*Mallotus villosus*) on the beaches around the province. The magnitude and implications of this problem, both to the whales and the fisherman, are assessed.

Key words: baleen whales, net entrapments, mortality assessment, capelin, *Balaenoptera acutorostrata*, *B. physalus*, *Megaptera novaeangliae*

PERKINS, J. S., AND P. C. BEAMISH. 1979. Net entanglements of baleen whales in the inshore fishery of Newfoundland. J. Fish. Res. Board Can. 36: 521–528.

On décrit dans cet article l'enchevêtrement de baleines dans les filets tendus dans les eaux côtières de Terre-Neuve et du Labrador. Les espèces concernées sont pour la plupart le petit rorqual (*Balaenoptera acutorostrata*) et la jubarte (*Megaptera novaeangliae*). Le rorqual commun (*Balaenoptera physalus*) se prend lui aussi dans les filets, bien que beaucoup plus rarement. Ces captures coïncident avec la fraie du capelan (*Mallotus villosus*) sur les plages de la province. Les auteurs évaluent l'amplitude et les conséquences de ce problème, tant pour les baleines que pour le pêcheur.

Received June 13, 1978
Accepted January 25, 1979

Reçu le 13 juin 1978
Accepté le 25 janvier 1979

A review of the impact of fishing techniques on mortality in smaller toothed Cetacea (odontocetes) has been outlined by Sergeant et al. (1970) and Mitchell (1975), but records of baleen whale (mysticete) entanglements in fishing gear are unusual (Table 1). Since 1974, we have been monitoring the incidence of whale entrapments, particularly those concerning baleen whales, in various types of fishing nets around the province of Newfoundland. One researcher sailed along the Newfoundland and Labrador coasts conducting field studies (Perkins and Whitehead 1977); the other studied some of the entrapped whales (Beamish 1978a). We collected data from firsthand sightings of whales in nets, strandings, interviews with fishermen, questionnaires, and newspaper articles. None of the information presented here was obtained from offshore vessels, and thus our sample represents only the inshore fishery in which fixed nets are used, specifically, cod traps, groundfish gill nets (strung in multiples and anchored near the bottom), and salmon nets (single gill nets positioned at the water's surface with one end fastened to shore).

In most cases, the whale swims into a net and either gets tethered by the anchor lines (Fig. 1), fouled in the netting itself, or both. Once tangled in a net, the whale may swim free breaking netting and lines, it may be cut loose, or it may die by drowning, by being killed, or by other unknown means. Whales are also reputed to swim through nets tearing large holes in the process, and although we have no direct evidence to support this claim, it seems a valid one. Most of the reported net entanglements have occurred in the summer months between mid-June and the end of August. All reports of animals not positively identified as whales have been discarded from the data presented in this paper.

Minke Whales, *Balaenoptera acutorostrata*

A data summary for minke whales is given in Table 2, which shows an annual entrapment of one to three whales ($\bar{X} = 2.6$) over a period of 5 yr. One whale, trapped in late June 1976, was not definitely identified as to species, but from fishermen's descriptions we suspect it to have been a minke whale and thus have included it to represent the maximum extreme of the data. The mortality rate for entrapped minkes is 0.77

[1]Present address: Ceta-Rescue/Research Inc., Trinity, Nfld. A0C 2S0.

Printed in Canada (J5275)
Imprimé au Canada (J5275)

TABLE 1. Records of baleen whale entanglements in fishing gear.

Date	Location	Species	Fishing gear	Fate
Aug. 20, 1856[a]	Lubec, Maine	minke	fish weir	killed
June 30, 1887[a]	Nantucket, Mass.	minke	blue-fish net	drowned
July, 1893[a]	Portland, Maine	minke	fish net	died
May 7, 1895[a]	Provincetown, Mass.	minke	fish weir	released alive
June 15, 1909[a]	Provincetown, Mass.	right	fish trap	killed by bomb lance
Aug. 21, 1909[a]	Provincetown, Mass.	minke	fish trap	died
May 18, 1910[a]	South Truro, Mass.	minke	fish trap	killed
June 25, 1910[a]	South Truro, Mass.	minke	fish trap	died
Aug. 14, 1910[a]	Provincetown, Mass.	minke	fish weir	killed
June, 1911[a]	Provincetown, Mass.	minke	fish trap	released alive
Feb. 25, 1975[b]	Cape Henry, Va.	humpback	gill net	died
July 1, 1976[c]	Long Branch, N.J.	right	lobster traps	released alive
Aug. 5, 1976[c]	Grand Manan, N.B.	right (cow & calf)	fish weir	released alive
Aug. 25, 1976[c]	Wellfleet, Mass.	right	netting	unknown

[a]Allen (1916).
[b]James Mead, Smithsonian Institution, Washington, personal communication.
[c]Reeves et al. (1977).

(10 out of 13). Eight of those that died were tangled in cod traps and two in gill nets. More minkes were caught in cod traps (10) than in other net types (2) and all were entrapped within a 3-mo period, between early June and late August (median July 7). Allen (1916) reports similar dates.

The majority of entrapments are concentrated on the east coast of Newfoundland (Fig. 2). Information on the lengths and sexes of trapped whales is mostly lacking, but three minkes were measured:

1) July 25, 1974, Cape Freels: 6.8-m male (measured by J.P. and P.B.); 2) June 23, 1976, Bear Cove: 7.4-m female (measured by J.P.); 3) June 23, 1977, Musgrave Harbor: 8.4 m (measured by Fisheries Officer J. Steele).

One minke whale was said by T. Lavallee, the fisherman who found it in his gill net near Lourdes du Blanc Sablon, Quebec, to have had a red ribbon-like plastic tag encircling the dorsal fin. Since the whale sank before the tag could be removed or photographed, we were not able to discover its origin.

FIG. 1. Humpback whale caught in a cod trap.

Humpback Whales, *Megaptera novaeangliae*

The data on humpback whales are summarized in Table 3. We have included two whales not positively identified, but suspected to be humpbacks, based on fishermen's descriptions. Of 19 whales reported entangled in nets during 7 yr, the range of annual entrapments was one to five with a mean of three whales. The mortality rate was 0.37 (7 out of 19), considerably lower than for minkes, with cod traps accounting for six deaths and gill nets for one death. Seven humpbacks got free of the nets; five of these were released and two escaped without help from humans. The fates of

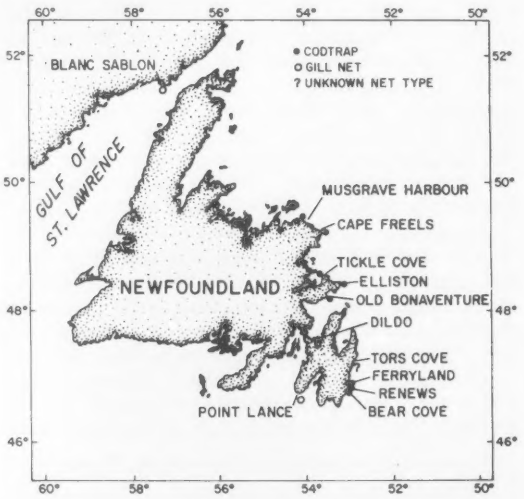


FIG. 2. Location of minke whale entrapments.

TABLE 2. Minke whale entrapments between 1973 and 1977 giving location, net type involved, and fate of the whales.

Date	Location		Net type[a]	Fate	Source[b]
1973					
?	Old Bonaventure	48°17′N, 53°26′W	ct	?	S.K.
?	Ferryland	47°01′N, 52°51′W	ct	drowned	J.P.
?	Tickle Cove	48°35′N, 53°29′W	ct	died	S.K.
1974					
July 25	Cape Freels	49°16′N, 53°29′W	ct	died	J.P.
Aug. 25	Salmon River, Lab.	?	ct	?	P.B.
?	Elliston	48°38′N, 53°02′W	ct	died	S.K.
1975					
July 3	Blanc Sablon, Que.	51°25′N, 57°06′W	gn	killed	J.P.
July 7	Pt. Lance	46°47′N, 54°04′W	gn	died	P.B.
July 27	Tors Cove	47°13′N, 52°51′W	?	?	P.B.
1976					
June 23	Bear Cove	46°53′N, 52°56′W	ct	died	J.P.
early June	Dildo	47°33′N, 53°34′W	ct	died	P.B.
late June[c]	Renews	46°56′N, 52°57′W	ct	died	J.P.
1977					
June 23	Musgrave Harbour	49°28′N, 53°57′W	ct	died	F.O.

[a]ct: cod trap; gn: gill net; ?: unknown net type.
[b]S.K.: Scott Kraus; J.P.: Judith Perkins; P.B.: Peter Beamish; F.O.: Fisheries Officer.
[c]Probable minke whale.

the other five entrapped humpbacks are unknown. Each of the three humpbacks released on June 23, 1975 (Beamish 1978a), June 30, 1976, and July 3, 1976 (Beamish 1978b) was tagged through the dorsal fin (by P.B.) with colored two-tailed polypropylene streamers having fringes at the trailing edges (Fig. 3). Although posters alerting people about these tagged humpbacks were distributed, none has been photographed since that time.

As with the minkes, the majority of humpbacks (12) were caught in cod traps compared with gill nets (2) and salmon nets (2). Most of the entrapments (Fig. 4) occurred between Joe Batt's Arm on Fogo Island (49°45′N, 54°09′W) and St. Brides near Cape St. Mary's (46°55′N, 54°11′W), nearly the same areas as minke whales. All the entrapments, with one exception on November 18, 1977, took place between June 10 and August 2 (median July 3), a period of <2 mo. The humpback found on November 18, 1977 was an unusually late entrapment. The net type was not specified, but the Fisheries Officer gave the following description: "... caught in a mooring at Dildo. Moorings included four 70 to 100 lb (32–45 kgm) graplens. One was cut free, but whale escaped with the remainder still entangled around it."

This whale was estimated to be 12 m long. Other records of lengths and sexes of entrapped humpbacks are as follows:

(1) June 30, 1972, Cavendish: 10 m (measured by J. Mead)

(2) August 2, 1973, Joe Batt's Arm: 10.7 m (measured by Fisheries Officer L. Freake)

(3) July 15, 1974, Joe Batt's Arm: 12.3 m (measured by Fisheries Officer L. Freake)

(4) June 23, 1975, Branch: 10.2-m male (measured by P.B.)

(5) June 30, 1976, Valleyfield: 9.2-m male (measured by P.B.)

(6) July 3, 1976, Elliston: 10.2-m female (measured by P.B.)

(7) June 17, 1977, Portugal Cove South: 12 m (estimate by Fisheries Officer)

(8) July 10, 1977, Plate Cove: 12 m (estimate by Fisheries Officer)

(9) July 31, 1977, Englee: 12 m (estimate by Fisheries Officer)

Only one instance of a cow and calf becoming entangled (June 17, 1975) has been reported.

Finback Whales, *Balaenoptera physalus*

Only one entrapped finback whale has come to our attention (Table 4). It swam into a salmon net off Spotted Island, Labrador (53°29′N, 55°45′W) and was apparently too active and cumbersome to be removed from the net. The fate of this whale is unknown.

Discussion

The mortality figures calculated from our data should be regarded as preliminary since our effort has been somewhat restricted. In 1977 questionnaires were distributed to Fisheries Officers throughout the province. Twenty-two of 100 distributed questionnnaires were re-

FIG. 3. Humpback whale tagged through its dorsal fin.

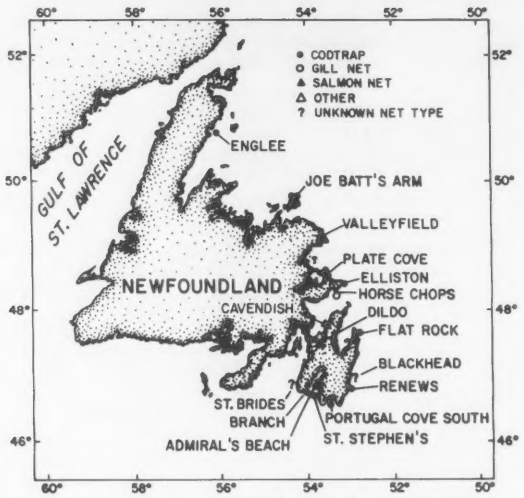


FIG. 4. Location of humpback whale entrapments.

turned, of which half contained information concerning specific baleen whale species. The questionnaires covered the entire spring through fall fishing season of 1977. At present, our figures show that, on average, approximately three minke, three humpback, and less than one finback whale become entrapped yearly and that of these approximately two minke, one humpback and less than one finback die every year as a result. But since our data contain a considerable number of entrapped whales whose fates are unknown, mortality rates could be significantly higher (Table 5). It should be noted that five humpbacks (26% of those entangled) were released alive, and that if humans hadn't intervened, the mortality figures could have been higher. Furthermore, it is likely that some net entanglements go unreported since there is a tendency for them to take place at the height of the capelin spawning season, when Atlantic cod (*Gadus morhua*) are most plentiful near shore. The fishermen, not wanting to lose valuable time, cut loose the whales and promptly resume fishing.

Although it appears that cod traps are responsible for more "catches" and deaths of minke and humpback whales than other net types, it is not possible to assess accurately the relative efficiency of different types of gear for "catching" whales, because the numbers of each net type set throughout the province have not been recently collected. Inshore fishing licenses are required for boats but not for nets, making this information hard to track down.

Cod traps are set out early in the season and are taken up normally by the end of August, primarily because the fish are no longer abundant close to shore. Jangaard (1974) notes that stomach contents of codfish collected near St. John's (47°34′N, 52°41′W) were analyzed by Templeman (1965) and revealed 98% capelin (*Mallotus villosus*), which, he says, is typical of codfish from Newfoundland's east and southeast coasts. To link whale entrapments to beach spawning capelin, we obtained dates of the first and last collections of these capelin in the St. John's area from 1966 through 1977, provided by the Fisheries' Biological Station in St. John's. These were compared to Templeman's (1948) maps which show dates when capelin spawn on Newfoundland's beaches for 1941 and 1942. The mean range of June 19–July 16 for the St. John's area corresponds well with Templeman's, indicating that his dates for beach spawning capelin throughout the rest of the province are probably valid today, even though there are local oscillations in the overall pattern. The ranges of these dates overlap with the cod trap season and are precisely those during which the majority

TABLE 3. Humpback whale entrapments between 1969 and 1977 giving location, net type involved, and fate of whales.

Date	Location		Net type[a]	Fate	Source[b]
1969					
June 18	St. Stephen's	46°45′N, 53°37′W	ct	killed	D.N. & P.B.
1972					
June 30	Cavendish	47°44′N, 53°31′W	sn	released alive	J.M.
1973					
?[c]	Plate Cove West	48°20′N, 53°30′W	ct	died	S.K.
Aug. 2	Joe Batt's Arm	49°45′N, 54°09′W	ct	?	F.O.
1974					
July 15	Joe Batt's Arm	49°45′N, 54°09′W	ct	?	F.O.
July 26	Flat Rock	47°42′N, 52°42′W	ct	died	J.P.
1975					
June 17[d]	Horse Chops	48°21′N, 53°13′W	gn	?	P.B.
June 19	St. Brides	46°55′N, 54°11′W	?	?	P.B.
June 23	Branch	46°52′N, 53°57′W	ct	released alive	P.B.
July 2	St. Brides	46°55′N, 54°11′W	gn	died	P.B.
Juy 28	Blackhead	46°59′N, 52°53′W	?	?	P.B.
1976					
Late June[e]	Renews	46°56′N, 52°57′W	ct	died	J.P.
June 30	Valleyfield	49°02′N, 53°42′W	sn	released alive	P.B.
July 3	Elliston	48°38′N, 53°02′W	ct	released alive	P.B.
1977					
June 10	Admiral's Beach	47°00′N, 53°39′W	ct	escaped	J.P.
June 17	Portugal Cove South	46°42′N, 53°16′W	ct	killed	F.O.
July 10	Plate Cove	48°30′N, 53′30°W	ct	escaped	F.O.
July 31	Englee	50°43′N, 56°07′W	ct	died	F.O.
Nov. 18	Dildo	47°33′N, 53°34′W	mooring	released alive	P.B.

[a]ct: cod trap; gn: gill net; sn: salmon net; ?: unknown net type.
[b]D.N.: The Daily News (St. John's); P.B.: Peter Beamish; J.M.: James Mead; S.K.: Scott Kraus; F.O.: Fisheries Officer; J.P.: Judith Perkins.
[c]Probably a humpback.
[d]Cow and calf; treated as one entrapment.
[e]Humpback or minke whale.

of minke and humpback whales become trapped in nets.

That minkes, humpbacks, and finbacks are following capelin is further substantiated by stomach content analysis from whales taken in Newfoundland waters. Mitchell (1973) found capelin in a majority of humpback stomachs that he examined, and Sergeant (1963) and Mitchell (1975) found capelin to be the primary food source of minke whales. Finback stomachs also revealed capelin as a primary prey item (Sergeant 1966; Mitchell 1974) along with euphausiid crustacea. Although all three species of whales feed heavily on capelin, it is likely that the low frequency of finback whale entrapment is due to their tendency to occur farther from shore than minkes or humpbacks (Perkins and Whitehead 1977).

The spawning of capelin begins earliest on Newfoundland's south coast, occurring progressively later farther north into Labrador, and this timing is due to their preference for spawning in water temperatures between 5.5° and 8.5°C (Jangaard 1974). Assuming that spawning densities are roughly the same from one beach to the next, we wondered whether the whales are moving progressively northward over the summer months. Figure 5 shows graphs of latitude vs. dates of

TABLE 4. Fin whale entrapment of 1975 giving location and net type.

Date	Location		Net type[a]	Fate	Source[b]
1975					
July 14	Spotted Island, Lab.	53°29′N, 55°45′W	sn	?	J.P. & P.B.

[a]sn: salmon net.
[b]J.P.: Judith Perkins; P.B.: Peter Beamish.

TABLE 5. Date summary of number of whale entanglements, showing fates and net types involved in both "catches" and deaths of the three species.[a,b]

	No. of minkes	No. of humpbacks	No. of finbacks
Net entanglements			
annual mean	2.6	2.7	<1.0
range	1–3	1–5	0–1
Net type involved in "catch"			
cod trap	10/13	12/19	0
gill net	2/13	2/19	0
salmon net	0	2/19	1/1
unknown	1/13	2/19	0
other	0	1/19	0
Fate of whales			
deaths	10/13	7/19	—
freed/escapes	0	7/19	1/1
unknown	3/13	5/19	0
Mean annual mortality (minimum)	2.0	1.0	—
Net type involved in deaths			
cod trap	8/10	6/7	0
gill net	2/10	1/7	0
salmon net	0	0	—
unknown	0	0	0

[a]These figures represent the maximum extreme of the data.
[b]Cow and calf pair are treated as one entrapment.

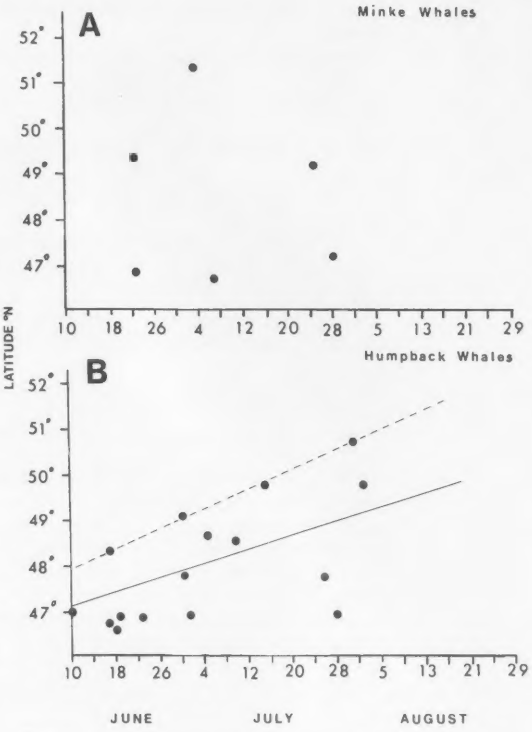


FIG. 5. A, Entrapment date vs. latitude for minke whales; B, entrapment date vs. latitude for humpback whales.

entrapment, when they are known. The minke whale data are inconclusive, but for humpbacks, the line that best fits the points seems to indicate a general movement into higher latitudes as the summer progresses. The correlation ($r = 0.575$; df = 14) is significant at the 2% level.

The dashed line is a regression through the four uppermost points which could represent the front of a migratory wave. The correlation ($r = 0.999$; df = 2) is highly significant (0.001%). The graph then can be interpreted as a northward movement plus continuing entrapment of residual animals that have stayed behind as the migration continues north.

We believe that because capelin is a principal food of both minke and humpback whales, there is a direct link between these whales' eating habits and the frequency of net entanglements. The impact of offshore capelin stocks being fished is not yet understood in terms of whether further decimation will affect the supply of food to whales and other animals. Catch statistics for offshore capelin in the waters around Newfoundland and the Grand Banks (ICNAF Division 3) show that in the 1960s the total catch for this division was on the order of 2000–3000 t/yr, and that by the mid 1970s it was up to over 200 000 t/yr (International Commission for the Northwest Atlantic Fisheries, Statistics Bulletin). During the capelin spawning season, large concentrations of humpbacks have been seen on the Grand Banks (J. Carscadden, Fisheries' Biological Station, St. John's, personal communication) and during various times of year, capelin are an important food source for other animals such as harp seals (*Pagophilus groenlandicus*) (Sergeant 1973) and many species of seabirds and fishes. Perhaps some of these whales must forage in inshore waters to find adequate food.

Whether the frequency of baleen whale entrapments has been increasing is not known. It may seem so, but this could be explained by increased observer effort. Another possible reason for an increasing entrapment rate would be that the whale populations are increasing; Canada's ban on whaling (late 1972) has afforded some protection to these whales. Very little is known about actual numbers of minke whales. They are reputed to approach ships (Beamish and Mitchell 1973; Christensen 1975; Mitchell 1975) which introduces a bias into censuses toward the high side.

There are two "aboriginal" whaling efforts presently taking humpbacks: one in Bequia, B.W.I., and a second in Greenland. Combined, they take not more than 10 whales a year (International Whaling Statistics 1955–68; Grønland 1971–74; Adams 1975) and usually fewer. Apparently these operations have not caused a

decline in the northwest Atlantic population, since it became internationally protected in 1955, for Allen (1970) suggests that this population of humpbacks may be increasing. Nevertheless, evidence for localized stocks, at least on the feeding grounds in the Gulf of Maine, is growing (Katona et al. 1978). Furthermore, there have been some winter sightings of humpbacks in Newfoundland waters: a sighting on November 18, 1977 in Trinity Bay (Fig. 4); an adult and calf sighted in March on the Grand Banks (Williamson 1961); and a group of five humpbacks confined to breathing holes in the ice of Hall's Bay (49°30′N, 56°05′W) during part of March and April 1978, suggesting that certain groups could be resident year round. Thus, deaths caused by net entanglements could have an impact on a particular stock of whales.

The locations of these entrapments may also provide some information about the distribution of minke and humpback whales. Because our personal efforts have been largely focused on Newfoundland's east coast, our sightings of these species on the west coast are minimal. We have seen no humpbacks along the west coast of Newfoundland and minkes only rarely, but along the east coast of Quebec minkes are fairly numerous during the months of June and July. Supportive evidence that minkes and humpbacks are scarce along the west coast of Newfoundland derives from the lack of reported entanglements for the Gulf of St. Lawrence, where cod traps and gill nets are also used during the summer months.

Although live whales can be released from nets successfully, we need to consider preventative measures as well. Playback experiments in which killer whale (*Orcinus orca*) "screams," between 200 and 5000 Hz, have been tested on gray whales (*Eschrichtius robustus*) by Cumming and Thompson (1971) and on belugas (*Delphinapteras leucas*) by Fish and Vania (1971) yielded positive results. In both cases, the whales changed their swimming direction, avoiding close contact with the "screams." However, when these sounds were played to southern right whales (*Eubalaena australis*) by Cummings et al. (1974), these whales showed no visible reaction. Possibly, since the killer whale "screams" were recorded in the Pacific northwest, they were inappropriate for southern hemisphere whales. Perhaps these whales are habituated to killer whales. The utility of such sounds in keeping away minke, humpback, and finback whales from fishing nets needs to be further tested. Submersible pingers have been found (by P.B.) to cause withdrawal of humpback whales; the pingers did not cause any reaction of either cod fish or capelin.

The net entrapment problem is a double-edged sword, for neither the whales nor the fishermen benefit from the experience. Economically, a whale in a cod trap can spell disaster. The most commonly used cod trap (18 m deep), complete with anchors and floats costs between $7000 and $8000 in 1978; gill nets and accessories cost ~$800–$4000, depending on the number of nets attached together; and a salmon net costs between $50 and $100. On top of this, one good cod trap haul may bring as much as $5000 at the peak of the season. Usually, nets involved in entanglements are not damaged beyond repair, but most of the time they need to be hauled for major mending jobs that can take several days. Thus, fixed fishing nets pose a threat of death to these whales, while whales in nets may represent severe financial losses to the inshore fishermen.

Acknowledgments

We especially thank H. Whitehead and S. Kraus for their help and support throughout the study, and L. Riche, J. Carscadden, R. Russell, the Fisheries Officers, the many people of Newfoundland and Labrador, and J. Mead for helping with our research. We also thank the Oceanic Society, the Ocean Research and Education Society, Ceta-Rescue/Research Incorporated, and the Canadian government for making the work possible.

ADAMS, J. E. 1975. Primitive whaling in the West Indies. Sea Frontiers 21: 303–313.

ALLEN, G. M. 1916. The whalebone whales of New England. Mem. Bos. Soc. Nat. Hist. 8: 222 p.

ALLEN, K. R. 1970. A note on baleen whale stocks of the northwest Atlantic. Rep. Int. Whaling Comm. 20: 112–113

BEAMISH, P. C. 1978a. Behavior and significance of entrapped baleen whales. *In* H. E. Winn and B. L. Olla [ed.] Behavior of marine animals. Vol. 3, Plenum Press, New York, N.Y.

1978b. Evidence that a captive humpback whale (*Megaptera novaeangliae*) does not use sonar. Deep-Sea Res. 25: 469–472.

BEAMISH, P., AND E. MITCHELL. 1973. Short pulse length audio frequency sounds recorded in the presence of a minke whale (*Balaenoptera acutorostrata*). Deep-Sea Res. 20: 375–386.

CHRISTENSEN, I. 1975. Norwegian whale marking in the northeastern North Atlantic in 1975. Int. Counc. Explor. Sea CM 1974/N: 19, 5 p.

CUMMINGS, W. C., AND P. O. THOMPSON. 1971. Gray whales, *Eschrichtius robustus*, avoid the underwater sounds of killer whales, *Orcinus orca*. Fish. Bull. U.S. 69: 525–530.

CUMMINGS, W. C., P. O. THOMPSON, AND J. F. FISH. 1974. Behavior of southern right whales: R/V *Hero* cruise 72-3. Antarct. J. March–April: 33–38.

FISH, J. F., AND J. S. VANIA. 1971. Killer whale, *Orcinus orca*, sounds repel white whales, *Delphinapteras leucas*. Fish. Bull. U.S. 69: 531–535.

GRØNLAND. 1971–74. Ministeriet for Grønland, Hausergade 3, 1129 København K.

INTERNATIONAL COMMISSION FOR THE NORTHWEST ATLANTIC FISHERIES, Statistical Bulletin. Dartmouth, Canada. Vol. 17 (1967)–26 (1976).

INTERNATIONAL WHALING STATISTICS. Oslo, Grøndahl and Son. No. 1 (1930)–64 (1968).

JANGAARD, P. M. 1974. The capelin (*Mallotus villosus*): biology, distribution, exploitation, utilization, and composition. Bull. Fish. Res. Board Can. 186: 70 p.

KATONA, S., B. BAXTER, O. BRAZIER, S. KRAUS, J. PERKINS, AND H. WHITEHEAD. 1978. Identification of humpback whales by fluke photographs. *In* H. E. Winn and B. L.

Olla [ed.] Behavior of marine animals. Vol. 3, Plenum Press, New York, N.Y.

MITCHELL, E. D. 1973. Draft report on humpback whales taken under special scientific permit by eastern Canadian land stations, 1969–1971. Rep. Int. Whaling Comm. 23: 138–154.

1974. Present status of northwest Atlantic fin and other whale stocks, p. 108–161. *In* W. E. Schevill [ed.] The whale problem. Harvard University Press, Cambridge, Mass.

[ed.] 1975. Review of biology and fisheries for smaller cetaceans. J. Fish. Res. Board Can. 32: 889–983.

PERKINS, J., AND H. WHITEHEAD. 1977. Observations on three species of baleen whales of northern Newfoundland and adjacent waters. J. Fish. Res. Board Can. 34: 1436–1440.

REEVES, R., J. MEAD, AND S. KATONA. 1977. The right whale, *Eubalaena glacialis*, in the western North Atlantic: a working paper submitted to the scientific committee of the IWC. Canberra: 1–27.

SERGEANT, D. E. 1963. Minke whales, *Balaenoptera acutorostrata* Lacépède. of the western North Atlantic. J. Fish. Res. Board Can. 20: 1489–1504.

1966. Populations of large whale species in the western North Atlantic with special reference to the fin whale. Fish. Res. Board Can. Circ. 9: 30 p.

1973. Feeding, growth and productivity of the northwest Atlantic harp seals (*Pagophilus groenlandicus*). J. Fish. Res. Board Can. 30: 17–29.

SERGEANT, D. E., A. W. MANSFIELD, AND B. BECK. 1970. Inshore records of Cetacea for eastern Canada, 1949–1968. J. Fish. Res. Board Can. 27: 1903–1915.

TEMPLEMAN, W. 1948. The life history of the capelin (*Mallotus villosus*, O. F. Müller) in Newfoundland waters. Bull. Nfld. Gov. Lab. 17: 1–151.

1965. Some instances of cod and haddock behaviour and concentrations in the Newfoundland and Labrador areas in relation to food. Int. Comm. Northwest Atl. Fish. Spec. Publ. 6: 449–461.

WILLIAMSON, G. R. 1961. Winter sighting of a humpback suckling its calf on the Grand Bank of Newfoundland. Nor. Hvalfangst-Tid. 50: 335–336, 339–341.

Studies on the Sediment of St. Margaret's Bay, Nova Scotia

T. G. HUGHES[1]

Department of Fisheries and Oceans, Marine Ecology Laboratory, Bedford Institute of Oceanography, Dartmouth, N.S. B2Y 4A2

HUGHES, T. G. 1979. Studies on the sediment of St. Margaret's Bay, Nova Scotia. J. Fish. Res. Board Can. 36: 529–536.

A detailed examination of the sediment in a small area of St. Margaret's Bay revealed it to be composed of two main types of particles, mineral particles and particles of organic debris. Debris was also adsorbed onto the mineral particles. Particles generally had relatively low numbers of bacteria associated with them indicating that most of the organic carbon in the sediment is refractory. Variation in size distribution of the component particles with depth in the sediment was found to be minimal, but a slight decrease in the proportion of debris particles was seen. Variation in size distribution along a transect was also limited, but where the mineral particles became coarser the size range of the debris particles remained constant. The relative amount of debris particles along the transect varied little but values for total organic carbon (by weight) changed dramatically.

Key words: sediment, particle composition, size distribution, organic carbon

HUGHES, T. G. 1979. Studies on the sediment of St. Margaret's Bay, Nova Scotia. J. Fish. Res. Board Can. 36: 529–536.

Un examen détaillé du sédiment dans une petite partie de la baie St. Margaret démontre qu'il est constitué de deux types principaux de particules, particules minérales et particules de débris organiques. Il y a aussi des débris absorbés dans les particules minérales. En général, les particules n'ont, associées avec elles, qu'un petit nombre de bactéries, ce qui indique que la plus grande partie du carbone organique dans le sédiment est réfractaire. Nous constatons que la variation dans la distribution des tailles des particules en fonction de la profondeur dans le sédiment est faible. Il y a par ailleurs légère diminution de la proportion des particules de débris. La variation dans la distribution des tailles le long d'une coupe transversale est également limitée, mais aux endroits où les particules minérales sont plus grosses, la gamme de tailles des particules de débris demeure constante. La quantité relative des particules de débris le long de la coupe transversale varie peu, mais les valeurs du carbone organique total (en poids) changent de façon dramatique.

Received June 8, 1978
Accepted January 31, 1979

Reçu le 8 juin 1978
Accepté le 31 janvier 1979

As part of a study of the ecology and biology of benthic polychaetes from St. Margaret's Bay, Nova Scotia, an examination of the sediment that forms the habitat and food for these animals was undertaken. Many studies (Bader 1954; Nichols 1970; Longbottom 1970; Ward 1975) have attempted to relate the distribution and abundance of benthic organisms to the characteristics of the sediment and although certain correlations are seen, the exact causes of these are not always apparent. Marine sediments are complex mixtures of particles (Meadows and Anderson 1968; Johnson 1974) but workers have tended to measure only one or two of the many parameters that characterize a sediment. One of these is usually the gravimetric determination of grain size (Krumbein and Pettijohn 1938; Buchanan 1971) where all the components are mixed, dried, and mechanically sieved. Devised by geologists, the technique concentrates on the mineral components and delicate but equally important organic components are damaged or broken down. Total organic carbon (TOC) is another frequently used measure but it gives no indication of the distribution of organic carbon amongst the particles or how available this is to organisms feeding on the sediment.

For these reasons observations were carried out on the various components of the St. Margaret's Bay sediment. Each was described and where possible spatial variations in their size distribution and relative abundance were estimated to see how these produce changes in the sediment. Many of the techniques used have not been widely applied to marine sediments and are merely experiments in viewing sediments at a different and more detailed level which is necessary if some of the factors controlling the distribution and abundance of benthic organisms are to be better understood.

[1]Present address: University of Khartoum, Saukin Marine Laboratory, P.O. Box 24, Port Sudan, Sudan.

Printed in Canada (J5273)
Imprimé au Canada (J5273)

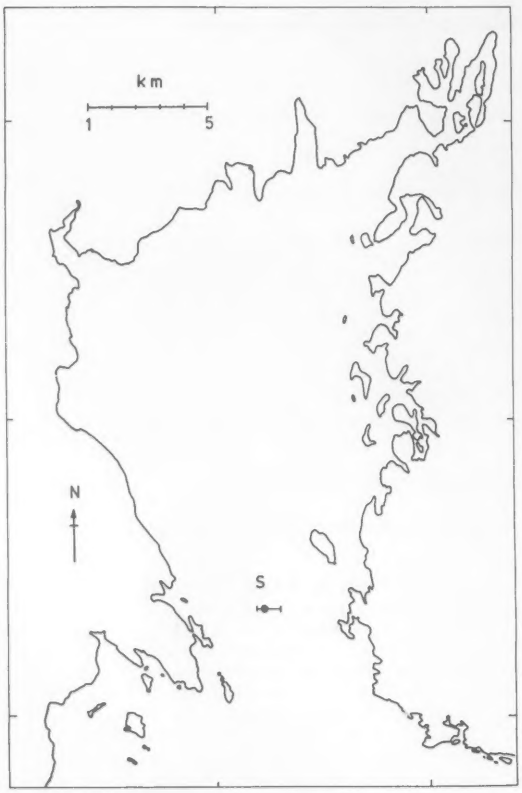


FIG. 1. St. Margaret's Bay, Nova Scotia, showing the mooring site (S) with the transect passing through it.

Materials and Methods

Material for this study was obtained from a small area of St. Margaret's Bay, Nova Scotia. Most samples were collected from a vessel secured to a permanent mooring located at Lat. 44°31.8′N, Long. 63°58.85′W in 70 m of water (Fig. 1). Although only a small area was sampled, the mooring was designed so that on successive sampling dates the vessel rested in a different position according to the direction and intensity of the wind and tide. Most samples were obtained with a van Veen grab, but when cores through the sediment column were needed, a gravity corer fitted with a 1.5 m length of 10 cm diam polyvinyl chloride piping was used. Samples of sediment were stored on ice following collection and then later frozen.

The topography of the surface of the sediment around the mooring was observed using a remotely controlled camera and flashgun set to make an exposure at a distance of 125 cm from the bottom. This gave a photograph of a portion of the bottom measuring 1.20 by 0.80 m.

Particles of the sediment were examined using bright-field and phase contrast microscopy. They were also stained to detect the presence of carbohydrates and proteins (Pearse 1960; Mazia et al. 1953). Bacteria and other microorganisms attached to particles could only be seen using fluorescent microscopy. For this, sediment held on a 0.80-μm millipore filter was fixed for 10 min in a 4% solution of formaldehyde made up in filtered seawater. After being rinsed in distilled water, it was transferred to an aqueous solution (1:600 000) of acridine orange for 7 min and then re-rinsed. The sediment could now be left overnight without any noticeable deterioration. A small portion of the stained sediment was removed from the filter, mounted in distilled water on a microscope slide, and viewed by transmitted UV light on a Carl Zeiss fluorescent microscope; a BG 12 exciter filter and a 53/44 barrier filter were employed.

The relative amount of organic debris adsorbed onto mineral particles was examined in particles of different sizes from different levels in the sediment. Samples of sediment from the surface and from a depth of 10 cm from the mooring area were graded into size groups of 25–50 μm and >153 μm. A sample from each of these groups was mounted on a microscope slide and the outlines of 50 particles and their adsorbed debris were traced onto paper. From this the relative surface area of the particle covered by debris was determined by planimetry. Only one aspect of any given particle could thus be viewed and measured and it was assumed that this gave a reliable estimate of the amount of debris adsorbed onto all the surfaces.

Particles were sized gravimetrically as outlined by Buchanan (1971) but particle size was usually determined by measuring the minimum dimensions of particles spread onto a microscope slide, thus allowing the different types of particles to be measured separately. Variation of size distribution with depth in the sediment was examined using samples from around the mooring site; samples were taken by gravity corer from the surface sediment and 10, 20, 30, 40, 60, 80, and 100 cm beneath this. The relative proportions of mineral and debris particles in these samples were determined by placing the particles in filtered seawater on a microscope slide and counting the number of mineral and debris particles (>10 μm in size) encountered along randomly selected traverses. For each sample, 10 determinations of the mineral:debris particle ratio were made and an average value calculated.

Samples were also taken along a transect line (Fig. 1) to investigate the variation of the size of the component particles and their relative proportions as the depth of the overlying water and the grain size of the sediment changes. Sampling stations were all 200 m apart and the depths of the water at each station were as follows: station 0, 68 m; station 1 (mooring site), 70 m; station 2, 64 m; station 3, 55 m; and station 4, 44 m. At each station two van Veen grab samples (A and B) were taken and from these subsamples were removed from the surface of the sediment and from 5 cm below it. Size and relative proportions of the component particles from these samples were determined under the microscope as before and, in addition, the total organic carbon content (TOC) (by weight) of the samples was estimated by the method of El Wakeel and Riley (1956).

Results

General Morphology of the Sediment

The surface of the sediment at the sampling stations was broken up by a variety of holes and depressions.

There were also tracks made by the more active members of the benthos and epibenthos suggesting that the substrate surface was relatively soft. Examination of sediment brought up in grab samples also showed that the upper 2–3 cm of sediment was relatively fluid and of a lighter color whereas the underlying sediment was always well compacted and black. The top 20 cm of sediment were permeated by numerous tubes made by various species of maldanid polychaetes. Some of the tubes protruded above the surface of the sediment.

Particle Composition

The sediment from this region of St. Margaret's Bay is composed of two main types of particles mixed in roughly equal proportions, those of mineral origin and those of organic origin. Figure 2A shows a typical array of the particles found at the sampling area.

A large proportion of the particles were pieces of "organic debris" (Fig. 2B). They generally have an amorphous appearance and gave a positive reaction to carbohydrate specific stains indicating their organic nature. A small number of particles showed a positive reaction to protein stains but these were generally fragments of plants or animals. Organic debris particles had minimum dimensions ranging from ~100 μm to <1 μm and, instead of being solid, appeared to be aggregations of smaller pieces. Many contained small mineral particles or diatom tests (Fig. 2B). Smaller organic debris particles were less dense and more fragile than larger ones.

Mineral particles ranged in size from >300 μm (minimum dimension) to <1 μm and the majority of them were angular in appearance (Fig. 2C) rating 0.30 on the roundness scale of Powers (1953). Particles <50 μm often appeared as flakes with smooth faces (Fig. 2D), the larger particles being more globular with uneven surfaces of many facets. Most of the mineral particles had varying amounts of organic debris adsorbed onto them, and bigger and more rounded particles carried the greatest load, although it was unusual to find a particle completely covered. Particles 10–50 μm appeared to have relatively small amounts of adsorbed debris and those <10 μm were invariably devoid of any visible debris. Organic debris adsorbed onto these particles was of the same appearance as that forming the discrete debris particles.

Microorganisms Associated with Sediment Particles

Only qualitative observations on microorganisms were made as direct cell counts proved too unreliable. Bacteria were the only group of microorganisms that were recognized on the surfaces of sediment particles.

Large debris particles were found to have fairly large numbers of bacteria aggregated into clumps and not spread evenly over the particle. With decreasing size, debris particles appeared to have an even greater number of cells per unit area but greater transmission of UV light by these particles could have caused more cells to be detected. Particles <25 μm appeared to have particularly high densities of bacteria but the cells tended not to be gathered into clumps. The highest densities were always found on the remains of a diatom or a fragment of macrophyte.

In contrast, small mineral particles, flakes <50 μm, were almost totally devoid of bacteria. These were also the mineral particles found to have the least amount of adsorbed debris. Only the larger, rougher, and less clean mineral particles had significant numbers of bacteria on them. A large mineral particle might have up to 20 clumps dotted over a visible surface with up to 15 or 20 cells in each clump. The clumps were usually associated with adsorbed debris but cover only a very small percentage of the total surface of the particle.

No differences were discernable in the abundance and distribution of bacteria on particles from the sediment surface and from a depth of 10 cm.

Organic Debris Adsorbed onto Mineral Particles

The amount of organic debris adsorbed onto mineral particles was expressed as the frequency with which certain levels of coverage occurred in the samples (Fig. 3). Large mineral particles (>153 μm) from the surface of the sediment generally carried considerable amounts of debris but variation between individual particles was great. Particles 25–50 μm had a significantly smaller proportion of their surfaces covered by debris (Kolmogorov–Smirnov test, one tailed, $P = 0.05$), a result that substantiates the microscopical observations on these particles.

Depth of the sediment appeared not to influence the levels of debris adsorbed onto mineral particles. Samples of particles >153 μm and 25–50 μm from a depth of 10 cm were not significantly different from their corresponding surface samples (Kolmogorov–Smirnov test, one tailed, $P = 0.05$).

Size Distribution of Sediment Particles

Gravimetric analysis of surface sediment from the mooring site showed it to have a silt content of 50.43%, a median particle diameter of ~60 μm, and a size distribution shown in Fig. 4.

Fig. 5A shows an example of the size distribution of debris and mineral particles in the surface sediment from the mooring site. Variation in size distribution with depth was determined from two cores and on comparing these no significant differences between them could be found (Kolmogorov–Smirnov test, two tailed, $P = 0.05$), indicating that they were representative. This applied to the debris particles as well as the mineral particles. Comparisons between samples of mineral particles from different levels along the cores showed that

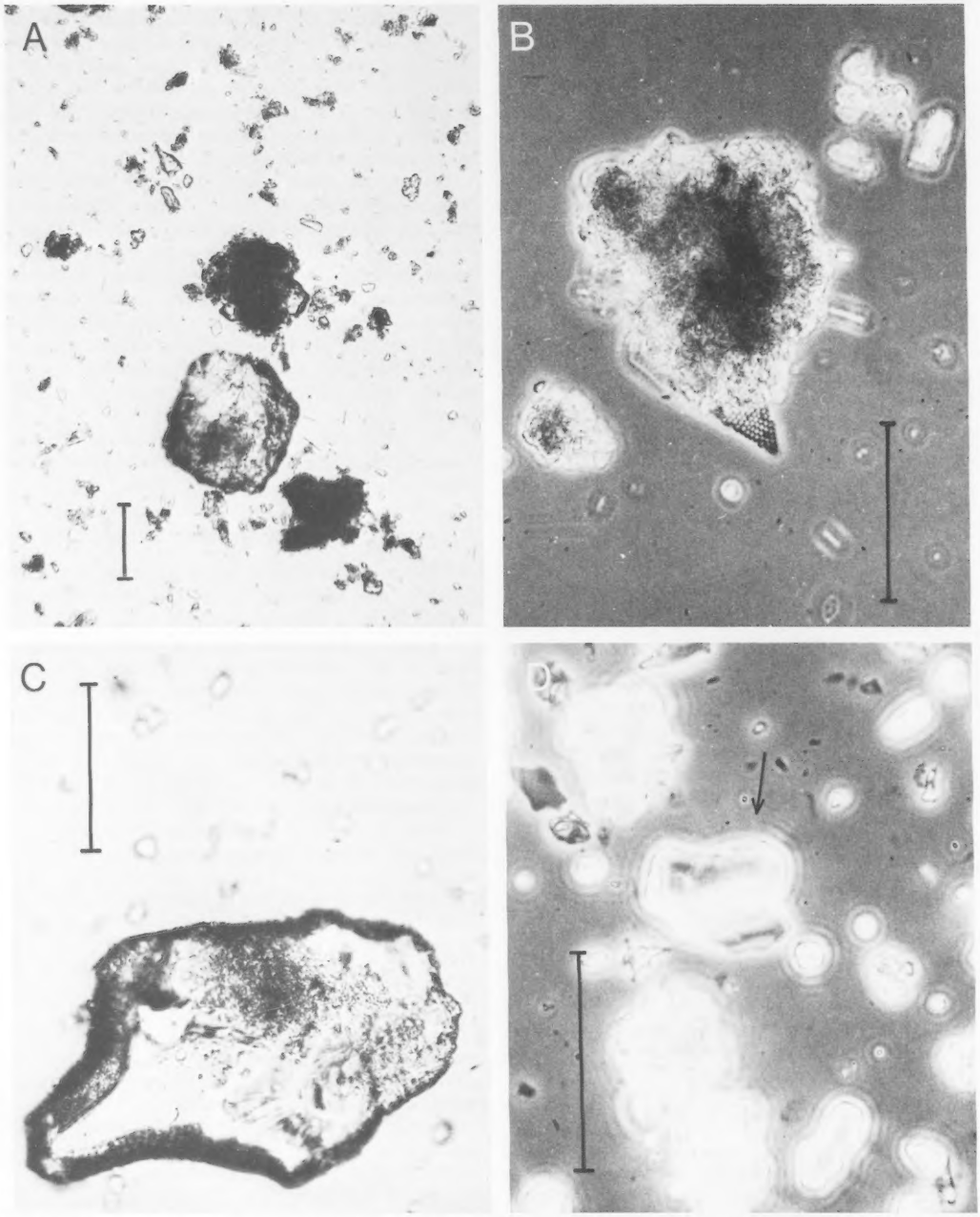


FIG. 2. A, Array of particles typical of the sediment of the study area. Brightfield illumination (bar = 100 μm). B, Debris particle of medium size. Phase contrast (bar = 50 μm). C, Large, rather angular mineral particle from the sediment of the mooring site. Some adsorbed debris can be seen on the upper surface. Brightfield illumination (bar = 100 μm). D, Small mineral flake (marked by arrow) that appears to be totally devoid of adsorbed material. Phase contrast (bar = 50 μm).

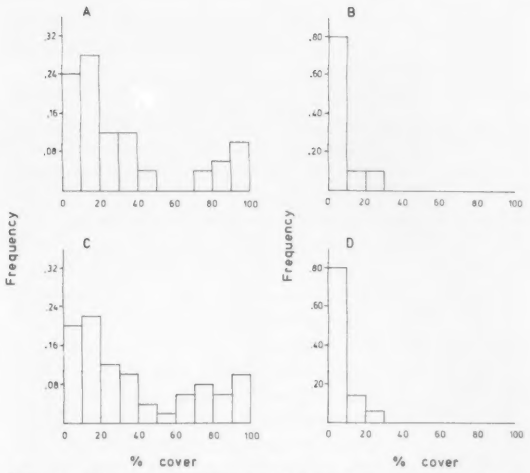


FIG. 3. Histograms showing the relative amounts of organic debris adsorbed onto mineral particles. A, >153-μm size group, surface sample; B, 25–50-μm size group, surface sample; C, >153-μm size group, 10-cm sample; D, 25–50-μm size group, 10-cm sample. For each of these distributions the sample size $n = 50$.

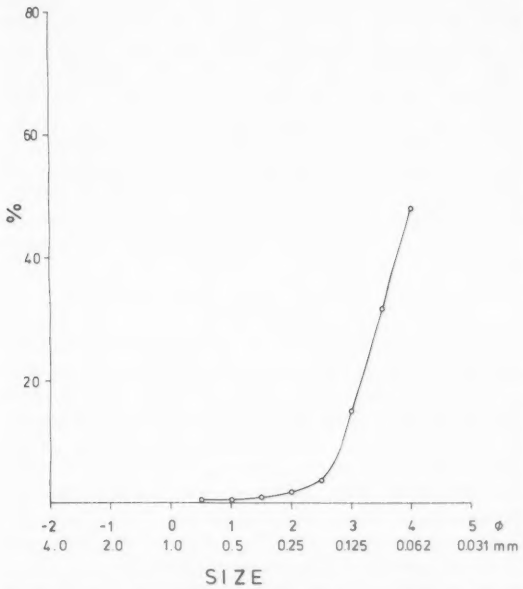


FIG. 4. Size distribution of the surface sediment at the mooring site determined by the gravimetric method. Cumulative frequency (%) is plotted against size (mm).

there were several that differed significantly from each other but they were spread throughout the samples and formed no discernable pattern. A similar situation

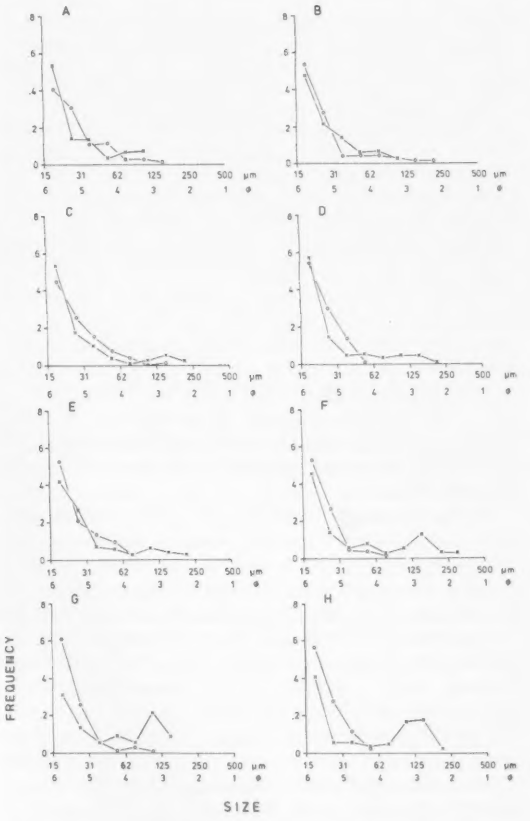


FIG. 5. Representative size distributions of mineral (×) and debris particles (○) from the stations along the transect. A, station 0 surface; B, station 0 5 cm depth; C, station 1 surface; D, station 1 5 cm depth; E, station 2 surface; F, station 2 5 cm depth; G, station 3 surface; H, station 3 5 cm depth.

existed with the debris particles and it seems likely that one or two samples deviating slightly from the rest gave rise to these discrepancies. Even when the mineral and debris particles from the cores were compared with each other they were found to have similar size distributions in most cases. The sediment column at the mooring site was thus relatively uniform with respect to the size distribution of the major components.

However, the mineral:debris particle ratios determined from one of the cores indicated a decrease in the relative abundance of debris particles with depth (Table 1). Surface sediment was significantly richer in debris particles than each of the seven levels beneath it (Mann–Whitney U test, one tailed, $P = 0.05$) and some of the subsurface levels were richer in debris particles than the level immediately below. However, although this gradient was detectable, it was by no means

TABLE 1. Core samples. Proportion of debris particles at different levels along the core (each represents the average of 10 determinations). Values marked * are not significantly different from each other. Others are all significantly different (Mann–Whitney U test, one tailed, $P = 0.05$).

Level	Total number of particles examined	Debris particles (%)
Surface	2387	66.2
10 cm	2381	60.3
20 cm	2389	56.0
30 cm	2398	50.4*
40 cm	2274	50.6*
60 cm	2410	56.3
80 cm	2332	48.9
100 cm	2282	51.7

drastic; the debris particles at the surface average 66% of the total compared with 49% at 80 cm, a difference of only 17%.

Of the five stations sampled along the transect, only four yielded good sediment samples. Station 4 had numerous large stones mixed with the sediment, causing the grab to remain partially open allowing most of the sediment to wash out. Representative size distributions of mineral and debris particles from each of the remaining four stations are given in Fig. 5. Comparisons between these samples were made using Kolmogorov–Smirnov test (two tailed, $P = 0.05$).

Comparisons between duplicate samples of mineral particles (the A and B samples) from the transect showed no significant differences indicating representative samples. Also, there was no difference between the size distribution of mineral particles at the surface and those at a depth of 5 cm, a result that agrees with results from the core samples. Mineral particles from stations 0, 1, and 2 were not significantly different from each other but samples from station 3 were all significantly different from those of the other stations. The difference was due to station 3 having a greater proportion of relatively large particles.

Similarly, debris particles of the duplicated transect samples were not significantly different except for the comparison 3A surface–3B surface. Between samples from the same station but from different levels the comparisons 1B surface–1B 5 cm and 3A surface–3A 5 cm differed significantly. However, on the whole, the samples were replicated well and depth variations in the size distributions were small. The few remaining differences followed no recognizable pattern, but there was no increase in the size range of debris particles at station 3 as shown by the mineral particles.

To complete the collation of particle size distribution along the transect, mineral particles were compared with debris particles. Mineral particles from station 3 were coarser than the debris particles but those from stations 0, 1, and 2 were generally of a similar size distribution to the debris particles. Thus the size distribution of debris particles from all the transect stations remained fairly constant irrespective of significant changes in the mineral particles.

TABLE 2. Average values of the proportion of debris particles and TOC (by weight) along the transect shown in Fig. 1 and described in the text.

Station	Debris particles (%)	TOC (%)
0A surface	59.5	3.93
0B surface	65.7	3.42
0A 5 cm depth	64.6	3.27
0B 5 cm depth	68.1	3.57
1A surface	70.5	2.18
1B surface	65.4	2.12
1A 5 cm depth	66.9	1.73
1B 5 cm depth	69.0	2.17
2A surface	64.5	1.42
2B surface	68.5	1.00
2A 5 cm depth	65.0	1.02
2B 5 cm depth	68.1	0.98
3A surface	61.7	0.55
3B surface	60.1	0.83
3A 5 cm depth	60.1	0.59
3B 5 cm depth	59.9	0.71

Finally, the variation in the mineral:debris particle ratio along the transect was examined. Duplicated samples were not significantly different from each other and only two instances of difference occurred amongst samples from stations 0, 1, and 2. Most discrepancies in the mineral:debris ratios occurred between samples of station 3 and those of stations 0, 1, and 2. Although significant, the actual magnitude of these differences (Table 2) was not very great. In contrast, a related parameter, TOC (by weight), was drastically reduced in passing from station 0 to station 3 (Table 2).

Discussion

The angular nature of the mineral particles of the sediment suggests that they have not been subjected to much abrasion (Powers 1953) and their high load of adsorbed debris indicates a stable sediment. However, this attached debris is not distributed uniformly; some particles have a greater covering of debris and among any size group there is still a wide variation between individual particles. Whether or not this reflects the history of particular particles is not known. The association of debris with mineral particles has also been reported by Steele and Baird (1968) and by Johnson (1974) in a survey of a wide range of sediments. The polychaete *Pectinaria gouldii* and the bivalve mollusc *Abra tenuis* can remove material adhering to sand grains (Whitlatch 1974; Hughes 1977) and it is probable that this is also a food source for many deposit feeding organisms. As such, it is useful to assess the amount of organic matter of sediments in this form

but the methods used here proved to be very time consuming and gave only relative values.

Another source of food, particles of organic debris (Darnell 1967; Tenore 1976), are also very common and they are similar to the organic–mineral aggregates described by Johnson (1974). Although there is no direct evidence to suggest the origin of these particles in St. Margaret's Bay, Webster et al. (1975) suggested that the majority of them were derived from the decay of macrophytes. Certainly the debris caught in the sediment traps used by Webster et al. (1975) is similar in appearance to that found in the sediment (M. A. Paranjape, personal communication).

Bacteria are closely associated with both forms of debris and are also potential food for macroorganisms. Intertidal sand grains examined by Meadows and Anderson (1968) showed the same kind of patchy distribution of bacteria as seen on those from St. Margaret's Bay, but the more abrasive environment of the intertidal zone seems to deplete particles of both microorganisms and debris. Wiebe and Pomeroy (1972) observed small aggregates of mineral and debris particles with low bacterial numbers in the open ocean but comparison with the present results are difficult due to the lack of quantification in both studies. Most debris particles from St. Margaret's Bay appear rather refractory in nature to judge by their low bacterial populations. The abundance of bacteria per unit area on these is relatively low when compared with particles of more recent origin (Gosselink and Kirby 1974). Bader (1954) has demonstrated that the amount of refractory carbon present is an important factor in determining the biomass sediments will support, but exactly how bacterial numbers relate to this is not clear. The picture is also confused by macroorganisms grazing on the bacteria (Fenchel 1971) and until direct counting techniques are refined so that the dynamics of bacterial populations can be followed, indirect measures such as ATP estimation (Ernst 1970; Karl and La Rock 1975) may prove more useful in assessing bacterial productivity.

Core samples from St. Margaret's Bay revealed that the sediment column in the study area was relatively uniform with respect to the size distribution of mineral and debris particles, in contrast to the situation described by Rhoads and Stanley (1965) and Rhoads (1967) where various polychaetes caused significant turnover and created stratification of the sediment column. The lack of stratification does not necessarily indicate a lack of sediment turnover in St. Margaret's Bay and all the subsequent studies on the fauna of this sediment suggests just the opposite. Probably the lack of vertical grading here is due to the sediment being much finer so that animals cannot select particles for ingestion on the basis of size. That debris particles raining down from the water column do not accumulate on the top of the sediment to any great extent but are distributed relatively evenly throughout the sediment column, also indicates a substantial turnover of sediment in St. Margaret's Bay. Johnson (1977) has also found that the sediment column in a variety of locations is relatively homogeneous with respect to particle type and organic content; he too, attributes this to the recycling of the sediment by benthic animals. In the present study there is a slight decrease in the proportion of debris particles with depth in the sediment column, but the reasons for this are not clear. A decrease in bioturbation with depth or the gradual breakdown of debris particles could be the cause. In any case, a substantial proportion of the debris particles still exists at a depth of 100 cm where little macrofaunal activity can be expected to occur and a lot of this carbon may not be used by the animals of the benthic community.

A sizeable area around the mooring site provides a uniform environment in terms of size distribution and relative proportions of the constituent particles. Only the most remote station sampled, station 3, showed any marked variation in the mineral component. The size distribution of the debris particles did not parallel this change but remained constant over the whole sampling area. If this debris is derived from the Bay's macrophytes, as suggested by Webster et al. (1975), only a certain size range would be suitable for transportation to the sampling site and the activity of the infauna would tend to further restrict it to a fairly constant size range. Relative proportions of debris particles are comparatively constant, although a slight reduction occurs at station 3. The reasons for this difference are not clear but it is slight and there would still appear to be considerable amounts of organic matter present.

Direct measurement of sediment particles has the advantage of allowing the component particles to be sized separately and without much disturbance to delicate organic particles which are important to sediment dwelling organisms (Bader 1954; Longbottom 1970). The method is slow and tedious but this could be overcome by using automated measuring techniques. Levels of organic matter in the sediment can also be assessed by direct observation and, although only relative values are obtained, under certain circumstances this gives a more accurate picture of the organic content than TOC. In the results from the transect, whereas TOC rapidly decreased towards station 3, the proportion of debris from direct counts remained relatively high. Because TOC is a function of weight, the increased density of the sediment towards station 3 causes the values to be depressed in spite of only minimal changes in the debris levels. Even if the amount of organic debris along the transect were to remain constant, TOC by weight would still decrease towards station 3. Situations do thus exist where assessment of the nutritive value of sediments from TOC as a function of weight can be quite misleading. However, both TOC and direct counts give no indication of how refractory the sediment carbon is and thus its usefulness to the infauna. Clearly the biological assessment of

sediments is a complex procedure and, although it is hoped that this paper illustrates some aspects of this, a thorough reevaluation of the methods used to examine this environment is still needed.

Acknowledgments

I am very grateful to Drs R. J. Conover and B. T. Hargrave for their criticisms of the manuscript.

BADER, R. G. 1954. The role of organic matter in determining the distribution of pelecypods in marine sediments. J. Mar. Res. 13: 32–47.

BUCHANAN, J. B. 1971. Sediments, p. 30–52. *In* N. A. Holme and A. D. McIntyre [ed.] Methods for the study of marine benthos. Blackwell Scientific Publications, Oxford and Edinburgh. I.B.P. Handbook No. 16.

DARNELL, R. M. 1967. Organic detritus in relation to the estuarine ecosystem, p. 376–382. *In* G. H. Lauff [ed.] Estuaries. Publ. Am. Assoc. Adv. Sci. 83.

EL WAKEEL, S. K., AND J. P. RILEY. 1956. The determination of organic carbon in marine muds. J. Cons. Perm. Int. Explor. Mer. 22: 180–183.

ERNST, W. 1970. ATP als Indikator für die Biomasse mariner Sedimente. Oecologia 5: 56–60.

FENCHEL, T. 1971. Aspects of decomposer food chains in marine benthos. Verh. Dtsch. Zool. Ges. 65: 14–23.

GOSSELINK, J. C., AND C. J. KIRBY. 1974. Decomposition of salt marsh grass, *Spartina alterniflora* Loisel. Limnol. Oceanogr. 19: 825–832.

HUGHES, T. G. 1977. The processing of food within the gut of *Abra tenuis*. J. Moll. Stud. 43: 162–180.

JOHNSON, R. G. 1974. Particulate matter at the sediment water interface in coastal environments. J. Mar. Res. 32: 313–330.

1977. Vertical variation in particulate matter in the upper twenty centimeters of marine sediments. J. Mar. Res. 35: 273–282.

KARL, D. M., AND P. A. LA ROCK. 1975. Adenosine triphosphate measurements in soil and marine sediments. J. Fish. Res. Board Can. 32: 599–607.

KRUMBEIN, W. C., AND F. J. PETTIJOHN. 1938. Manual of sedimentary petrography. Appleton-Century-Crofts, Inc., New York, N.Y. 594 p.

LONGBOTTOM, M. R. 1970. The distribution of *Arenicola marina* (L.) with particular reference to the effect of particle size and organic matter in the sediment. J. Exp. Mar. Biol. Ecol. 5: 138–157.

MAZIA, D., P. A. BREWER, AND M. ALFERT. 1953. The cytochemical staining and measurement of protein with mercuric bromophenol blue. Biol. Bull. 104: 57–67.

MEADOWS, P. S., AND J. G. ANDERSON. 1968. Micro-organisms attached to marine sand grains. J. Mar. Biol. Assoc. U.K. 48: 161–175.

NICHOLS, F. H. 1970. Benthic polychaete assemblages and their relationship to the sediment in Port Madison, Washington. Mar. Biol. 6: 48–57.

PEARSE, A. G. E. 1960. Histochemistry. J. and A. Churchill. 998 p.

POWERS, M. C. 1953. A new roundness scale for sedimentary particles. J. Sediment Petrol. 23: 117–119.

RHOADS, D. C. 1967. Biogenic reworking of intertidal and subtidal sediments in Barnstable Harbour and Buzzards Bay, Massachusetts. J. Geol. 75: 461–476.

RHOADS, D. C., AND D. J. STANLEY. 1965. Biogenic graded bedding. J. Sediment. Petrol. 35: 956–963.

STEELE, J. H., AND I. E. BAIRD. 1968. Production ecology of a sandy beach. Limnol. Oceanogr. 13: 14–25.

TENORE, K. R. 1976. Detrital utilization by the polychaete, *Capitella capitata*. J. Mar. Res. 33: 261–274.

WARD, A. R. 1975. Studies on the sublittoral free-living nematodes of Liverpool Bay. II. Influence of sediment composition on the distribution of marine nematodes. Mar. Biol. 30: 217–225.

WEBSTER, T. J. M., M. A. PARANJAPE, AND K. H. MANN. 1975. Sedimentation of organic matter in St. Margaret's Bay, Nova Scotia. J. Fish. Res. Board Can. 32: 1399–1407.

WHITLATCH, R. B. 1974. Food resource partitioning in the deposit feeding polychaete *Pectinaria gouldii*. Biol. Bull. 147: 227–235.

WIEBE, W. J., AND L. R. POMEROY. 1972. Micro-organisms and their association with aggregates and detritus in the sea: a microscopical study. Mem. Ist. Ital. Idrobiol. (Suppl. 29): 325–352.

Predation of Sea Scallops, *Placopecten magellanicus*, by the Rock Crab, *Cancer irroratus*, and the American Lobster, *Homarus americanus*

ROBERT W. ELNER AND GLEN S. JAMIESON[1]

Department of Fisheries and Oceans, Biological Station, St. Andrews, N.B. E0G 2X0

ELNER, R. W., AND G. S. JAMIESON. 1979. Predation of sea scallops, *Placopecten magellanicus*, by the rock crab, *Cancer irroratus*, and the American lobster, *Homarus americanus*. J. Fish. Res. Board Can. 36: 537–543.

Various predation techniques used by rock crabs, *Cancer irroratus*, and American lobsters, *Homarus americanus*, to open sea scallop, *Placopecten magellanicus*, prey are reported. Adult rock crabs and lobsters successfully fed on sea scallops up to 72 and 76 mm in height, respectively. Juvenile lobsters were capable of preying on scallops whose height exceeded their own carapace length. Daily predation rates on a range of scallop sizes by various sizes of predator were obtained. Given a choice, both rock crabs and lobsters showed distinct and constant preferences for certain size scallops. This preferred prey size increased with predator size. These laboratory studies demonstrated that both rock crabs and lobsters are potential scallop predators.

Key words: sea scallops, crabs, lobsters, predation techniques, prey selectivity

ELNER, R. W., and G. S. JAMIESON. 1979. Predation of sea scallops, *Placopecten magellanicus*, by the rock crab, *Cancer irroratus*, and the American lobster, *Homarus americanus*. J. Fish. Res. Board Can. 36: 537–543.

Nous décrivons diverses méthodes de prédation employées par les crabes-tourteaux communs, *Cancer irroratus*, et les homards d'Amérique, *Homarus americanus*, pour ouvrir les pétoncles géants, *Placopecten magellanicus*. Les crabes adultes et les homards réussissent à manger des pétoncles de tailles allant jusqu'à 72 et 76 mm de hauteur respectivement. Les jeunes homards peuvent exercer leur prédation sur des pétoncles dont la hauteur dépasse leur propre longueur de carapace. Nous avons déterminé les taux quotidiens de prédation sur une gamme de tailles de pétoncles par des prédateurs de diverses grosseurs. S'ils ont le choix, les crabes-tourteaux et les homards exhibent tous deux des préférences distinctes et uniformes pour des pétoncles d'une certaine taille. Cette taille de proie préférée augmente en fonction de la taille du prédateur. Ces études de laboratoire démontrent que le crabe-tourteau commun et le homard d'Amérique sont tous deux d'éventuels prédateurs du pétoncle géant.

Received October 25, 1978
Accepted January 26, 1979

Reçu le 25 octobre 1978
Accepté le 26 janvier 1979

THE sea scallop, *Placopecten magellanicus*, supports a major fishery in eastern Canada and the USA. Little is known, though, about the causes of its natural mortality. Caddy (1968, 1973) identified rock crabs, *Cancer irroratus*, as well as starfish, *Asterias vulgaris*, and several species of groundfish (*Myoxocephalus* sp., winter flounder, *Pseudopleuronectes americanus*) as probable predators of scallops damaged during trawling and dredging operations. Starfish (*A. vulgaris, Crossaster papposus*), Atlantic cod (*Gadus morhua*), American plaice (*Hippoglossoides platessoides*), and Atlantic wolffish (*Anarhichas lupus*) have been reported as natural scallop predators by Medcof and Bourne (1964). Dickie and Medcof (1963) regarded temperature fluctuations as a major cause of scallop mortality in the southwestern Gulf of St. Lawrence and cited starfish (*Asterias vulgaris*) and moonsnails (*Lunatia heros*) as natural predators that were able to capitalize on scallops made vulnerable by temperature debility.

The feeding behavior, predatory capabilities, and foraging strategies of both the rock crab and the American lobster are still largely undefined. Most studies have been performed in relation to the hypothesis that lobsters are "keystone" predators in controlling sea urchin, *Strongylocentrotus droebachiensis*, populations (Breen and Mann 1976; Mann 1977; Mann and Breen 1972). However, both rock crabs and lobsters are abundant in areas where scallops occur naturally (Caddy 1973; Scarratt 1975), and stomach analyses of rock crabs (Scarratt and Lowe 1972) and lobsters (Ennis 1973; Weiss 1970) show that bivalves form a significant part of the diet of both species. Furthermore,

[1]Present address (G.S.J.): Department of Fisheries and Oceans, Invertebrates and Marine Plants Division, Resource Branch, Halifax, N.S. BEJ 2S7.

Printed in Canada (J5412)
Imprimé au Canada (J5412)

Squires (1970) found that 3% (5) of 182 lobster stomachs examined from Port au Port Bay and Bay of Islands, Newfoundland, contained scallop remains. Rock crabs and lobsters are considered opportunistic omnivores, and, although food preferences can be demonstrated (Evans and Mann 1977; Hirtle and Mann 1978), stomach analyses suggest that the animals' diets largely reflect the relative abundance of prey species available in their immediate habitat. Given, therefore, that rock crabs and lobsters are potentially significant predators of scallops in nature, laboratory experiments were performed to investigate the techniques used by rock crabs and lobsters to break open scallops, the frequency of predation on a range of scallop size-classes by specified size-classes of rock crabs and lobsters, predation by rock crabs and lobsters on a single preferred size-class of scallops, predation on scallops by juvenile lobsters, and the maximum size of scallop vulnerable to selected sizes of rock crabs and lobsters.

Materials and Methods

Rock crabs and lobsters were captured by otter trawl in Passamaquoddy Bay, New Brunswick, near the mouth of the Bay of Fundy. During experiments animals were kept individually in 50 × 35-cm glass aquaria filled to a depth of 25 cm with running seawater at 12 ± 1°C. Scallops were dredged from Passamaquoddy Bay using a Digby tumble drag.

Rock crab size was determined by measuring the carapace width between the tips of the most distal marginal teeth. We determined lobster size by measuring carapace length from the posterior edge of the eye socket to the posterior edge of the carapace parallel to the longitudinal axis. Scallop size, in terms of height, was measured from the middle of the hinge across to the farthest shell edge.

Predators were starved for 2 d before feeding experiments to standardize hunger levels. We used only undamaged, healthy scallops, less than a week in captivity, as prey.

Predation techniques of adult rock crabs from 90 to 130 mm carapace width and lobsters from 70 to 140 mm carapace length on scallops 5–80 mm height were observed. We also recorded the techniques used by juvenile lobsters 6.3–42.2 mm carapace length to open small scallops 5–35 mm height. Shell fragments were collected after each successful attack to aid in the interpretation and description of the breaking techniques.

Individual rock crabs and lobsters of two size-classes, each size-class being composed of four individuals, were presented with five size-classes of scallops, each represented by five individuals scattered over the floor of the tank.

Prey sizes (mm)
Scallops (height): 20–30, 30–40, 40–50, 50–60, 60–70
Predator sizes (mm)
Lobsters (carapace length): 70–80, 130–140
Rock crabs (carapace width): 90–100, 120–130

The number of scallops eaten in each size-class was monitored daily for 5 d, any scallops eaten being replaced by ones of a similar size to maintain prey availability. We used only male crabs and lobsters to avoid potential bias caused by sexual differences in morphology and predation behavior.

Three male crabs of 120–130 mm carapace width and four male lobsters of 130–140 mm carapace length were fed 15, 40–50 mm height scallops daily for 7 d. Any scallops eaten were replaced daily. The size of scallops was chosen to be near that previously demonstrated as being preferred by both size-classes of predators.

Thirty-three juvenile lobsters, of both sexes, 6.3–42.2 mm carapace length were each presented with a scallop of between 20 and 25 mm height. We then replaced scallops that were eaten with prey from successively larger 5-mm size-classes. Experiments were continued until each lobster had had an unopened scallop in its tank for 5 d.

Male lobsters of between 70 and 150 mm carapace length and male rock crabs 90–132 mm carapace width were given successively larger scallops to prey upon; a critical, upper threshold of prey size was considered to have been reached when the scallop remained unopened for 5 d.

Results

The following distinct opening techniques were recorded for rock crab and lobster predation on scallops: In all cases the scallops were manipulated at some stage by the predator's mouthparts and anterior walking legs. Rock crabs tore away flesh using their chelae and mouthparts after cracking the shell. Lobsters used only their mouthparts to glean flesh from the broken shell. Invariably the shell would be broken into smaller fragments by both rock crabs and lobsters and torn apart before all the flesh was extracted. Specific shell-opening techniques used by the two predator species were as follows:

Rock crabs readily crushed outright small scallops up to ~10 mm in height. The prey was held by one of the chelae and indiscriminately crushed by the other chela before being passed to the mouthparts.

Hinge crushing was the commonest and most successful scallop-opening technique employed by rock crabs on scallops between 10 and 60 mm in height. The scallop was held by one chela while the other chela crushed the shell about the hinge in a lateral direction. Occasionally crabs were observed breaking smaller sizes of scallops by crushing the hinge in a dorsoventral direction. Once the hinge was broken, the scallop was left gaping and defenseless (Fig. 1).

Rock crabs were often observed attacking the margins of larger scallops, usually >50 mm in height, which could not be crushed. Pieces of shell were pried and chipped away by the chelae from the prey edge until a dactylus tip could be wedged between the scallop shell valves. Each valve was then grasped by a chela and the two valves were torn apart to expose the flesh. Successful attacks were invariably directed towards the vulnerable hinge region, where the inserted dactylus tip could damage the critical adductor muscle. However, overall, this method seemed to be the least productive of the three scallop predation techniques used by rock crabs.

Adult lobsters appeared to make more use of their relatively larger mouthparts and less of their chelae

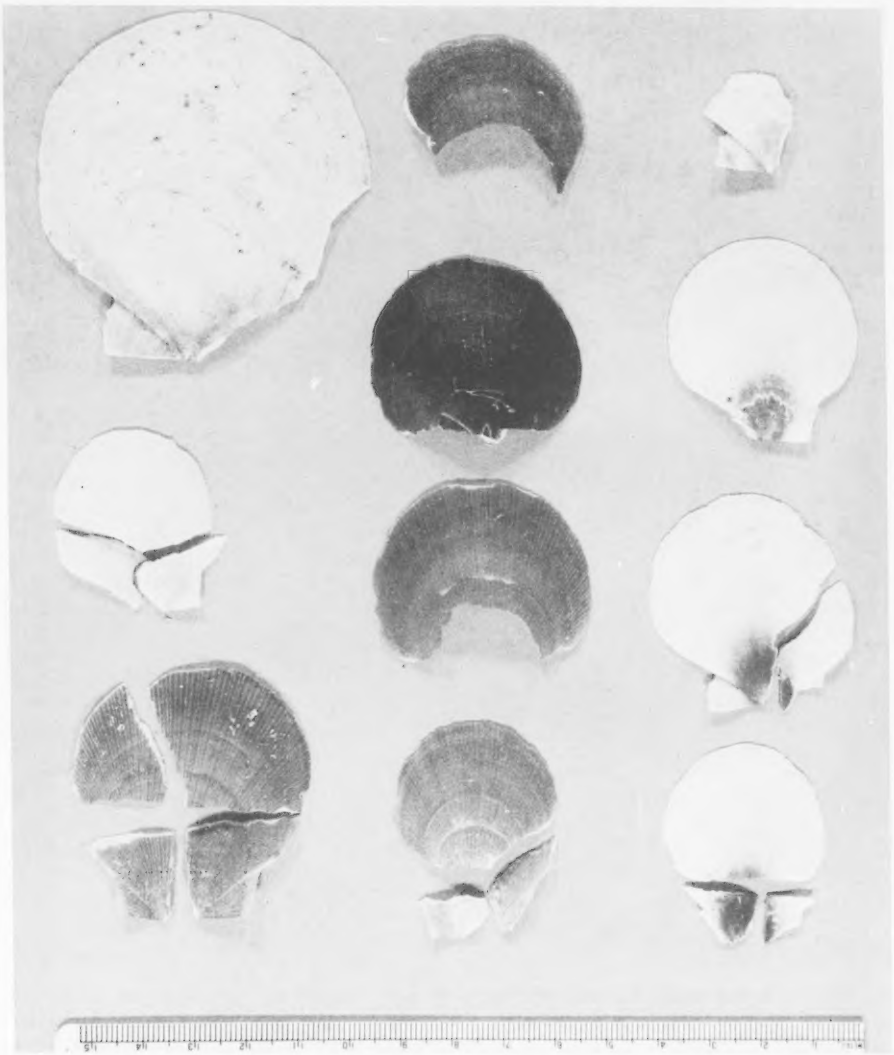


FIG. 1. Shell remains from sea scallops opened by adult rock crabs. Note the largely hinge-oriented attack.

than rock crabs in opening scallops. Lobsters crushed small scallops of ~10 mm in height indiscriminately by using their mouthparts alone. Large scallops up to ~70 mm height were crushed by the chelae in the lateral plane. No part of the scallop shell appeared to be attacked preferentially. Usually the lobster's slender cutter chela served to hold the prey whilst the more robust crusher actually cracked the shell (Fig. 2).

Lobsters were frequently observed biting away at the margins of larger scallops, using only their mouthparts. This opening method was rarely successful, however, and was frequently followed by another attack technique before the scallop was opened.

Juvenile lobsters were more specific than adults in their opening techniques and usually used only their chelae in attacking scallops. Scallops were most frequently crushed in a lateral direction about the hinge (Fig. 3). Scallop shells preyed upon by juvenile lobsters and rock crabs are characterized by the hinge-oriented attack.

Adult rock crabs and lobsters of the sizes used were able to open successfully and eat the full range of sizes

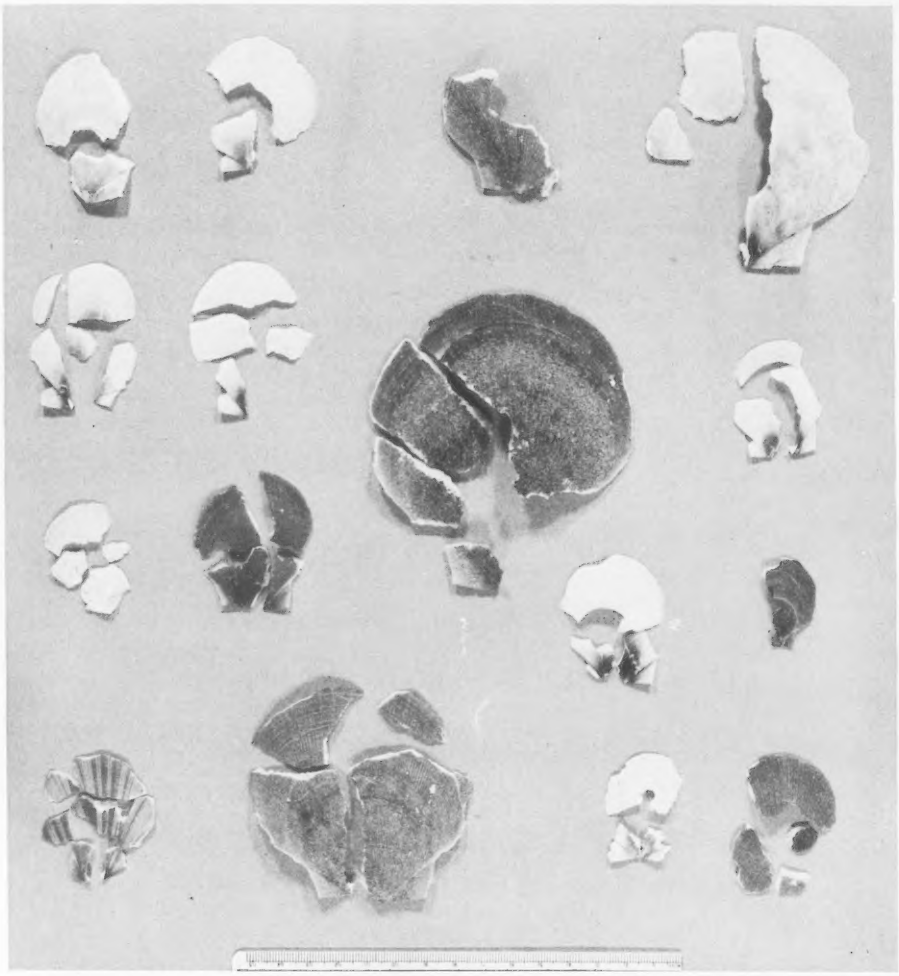


FIG. 2. Scallop shell fragments resulting from adult lobster predation. Note the more varied shape of the fragments, compared with rock crab attack, resulting from a more indiscriminate crushing.

of scallops presented to them. However, both rock crabs and lobsters demonstrated preferences for specific size-classes of scallops (Fig. 4). Moreover, larger size-classes of rock crabs and lobsters showed a preference for larger scallops than the smaller predators. Feeding rates were variable both between predators of the same size-class and individual rock crabs and lobsters from day to day (Fig. 5). The mean daily predation rate (±SE), on the preferred size-class of scallops, for rock crabs of 120–130 mm carapace width was 5.86 ± 0.85 scallops per day, and for lobsters of 130–140 mm carapace length 6.07 ± 0.67 scallops per day (Table 1).

The smallest juvenile lobster able to successfully break open a 20–25 mm height scallop was 20.8 mm in carapace length. The smallest juvenile lobsters to open 25–30 and 30–35 mm height scallops were 25.0 and 29.0 mm in carapace length, respectively. None of the juvenile lobsters between 6.3 and 42.2 mm carapace length was able to open a scallop >35 mm in height.

One of the largest rock crabs, a male of 130.4 mm carapace width, successfully ate a scallop 72 mm in height. Two of the largest available male lobsters of 130.9 and 147.6 mm carapace length were able to break open scallops of 74 and 76 mm height, respectively. No other rock crabs or lobsters surpassed these results in this experimental series.

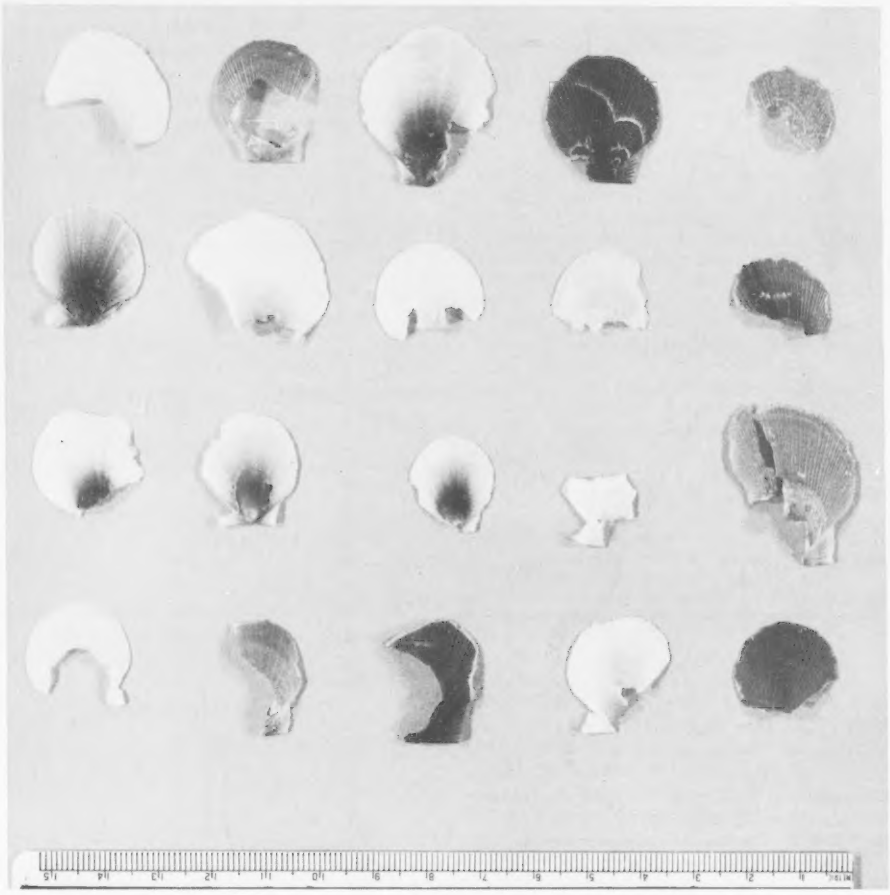


FIG. 3. Shell remains from scallops opened by juvenile lobsters. Note hinge-oriented attack.

Discussion

Rock crabs and lobsters had a variety of scallop-opening techniques that varied with prey size and shell strength. Rock crabs crushed smaller weaker-shelled scallops outright, whereas larger prey sizes required more sophisticated opening techniques, which usually centered on the scallop's vulnerable hinge region. Less frequently, crabs were observed to pry and tear the scallop valves apart. Adult lobsters showed less specific opening techniques than rock crabs and juvenile lobsters, probably because they possess larger, more powerful chelae that render the whole scallop (of the sizes used) vulnerable to crushing. However, notwithstanding their small chelae and smaller size, rock crabs appeared to be equivalent predators to lobsters, opening prey almost as large at a comparable rate. It is therefore possible, despite the fact that lobsters and rock crabs are known to prey on each other (Elner unpublished data), that these predators would be in direct competition for a limited prey resource on scallop beds.

Rock crabs and lobsters were able to feed on scallops from a wide size range, at rates proportional to the relative predator:prey size ratio. Both the rock crab and lobster showed distinct and constant preferences for certain scallop sizes and this preferred prey size increased with predator size. Elner and Hughes (1978) detailed similar prey size selective behavior in the green crab, *Carcinus maenas*, preying on mussels, *Mytilus edulis*. *Carcinus maenas* was found to be foraging optimally by maximizing its energy intake (prey flesh consumed per unit foraging time) by actively selecting prey. Selective predation strategies also reduce intraspecific competition for a limited prey resource by partitioning prey amongst the predator population. Whether the rock crabs' and lobsters' prey selectivity is adapted in the same way as the green crabs' remains to be investigated. Likewise, the active behavioral or

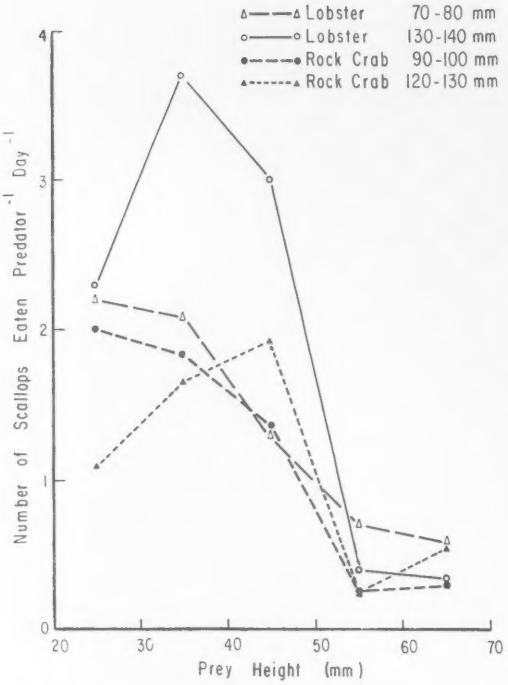


FIG. 4. Mean daily scallop consumption (±SE) plotted against scallop height, for two rock crab and two lobster size-classes.

TABLE 1. Daily feeding rates (±SE) for crabs and lobsters presented with 15, 40–50 mm height scallops per day for 7 d.

Predator and size	Numbers of scallops eaten/d: Day of experiment 1	2	3	4	5	6	7	Mean daily feeding rate
Lobster								
139.9 mm	7	4	5	8	9	9	10	7.42±0.84
134.8 mm	8	10	12	11	11	10	9	10.14±0.51
135.7 mm	4	4	2	3	2	3	3	3.00±0.31
137.6 mm	1	2	4	1	4	4	10	3.71±1.17
Mean daily feeding rate (±SE) = 6.07±0.67								
Rock crab								
129.7 mm	6	11	1	12	2	9	2	6.14±1.74
124.1 mm	4	4	6	5	4	6	0	4.14±0.77
121.4 mm	10	11	3	11	3	2	11	7.29±1.64
Mean daily feeding rate (±SE) = 5.86±0.85								

passive mechanical mechanism behind the prey selectivity of rock crabs and lobsters has also to be determined.

Rock crabs of 120–130 mm carapace width, and lobsters of 130–140 mm carapace length, which were

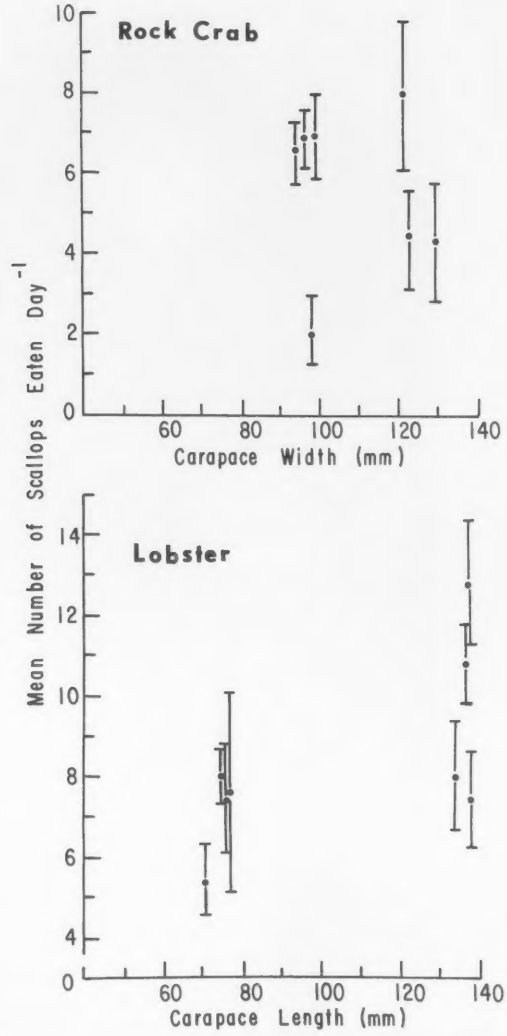


FIG. 5. Individual rock crab and lobster feeding rates, expressed as mean number (±SE) of scallops eaten per day relative to predator size.

fed only scallops from their previously demonstrated preferred size-classes, attained mean daily predation rates (±SE) of 5.86 ± 0.85 and 6.07 ± 0.67 scallops per day, respectively. Lobster and rock crab predation rates on a diet of scallops over the whole range of size-classes studied were in the same order, although feeding rates were variable. These results can be compared with those cited by Dickie and Medcof (1963) for laboratory experiments conducted by Chiasson (unpublished data). In these experiments various sizes of starfish, *A. vulgaris*, generally believed to be a major scal-

lop predator, ate a range of scallop sizes at a mean rate of 0.02 scallops per starfish per day.

That smaller scallops are particularly vulnerable as prey was demonstrated by the ability of even very small juvenile lobsters to successfully feed on scallops larger than their own carapace length. Scallops larger than a critical size, ~70 mm height, seemed immune to predation by comparatively large rock crabs and lobsters.

It remains to be seen if these laboratory studies, albeit where the scallops were not afforded full opportunity to utilize their normal swimming escape response, are relevant to the field situation. Recent investigations by Elner (1978), Krantz and Chamberlin (1978), and Whetstone and Eversole (1978) have demonstrated that green crabs, *C. maenas*, blue crabs, *Callinectes sapidus*, and mud crabs, *Panopeus herbstii* are well adapted and voracious predators of commercially valuable bivalves other than scallops. Previous studies on scallop population dynamics (Dickie 1955; Merrill and Posgay 1964) have not considered specific predators and have estimated natural mortality from "clucker" numbers (empty scallop valves still attached by the hinge). If these laboratory observations represent the field situation, and rock crabs and lobsters are significant predators of small- and intermediate-sized scallops, then scallop mortality estimates from "cluckers" alone are likely to be low since both rock crabs and lobsters invariably crush or separate the valves.

Acknowledgments

We are grateful to Drs A. Campbell, D. F. Markle, and K. G. Waiwood for helpful criticism of the manuscript. P. W. G. McMullon and F .B. Cunningham prepared the figures.

Breen, P. A., and K. H. Mann. 1976. Changing lobster abundance and the destruction of kelp beds by sea urchins. Mar. Biol. 34: 137–142.

Caddy, J. F. 1968. Underwater observations on scallop (*Placopecten magellanicus*) behaviour and drag efficiency. J. Fish. Res. Board Can. 25: 2123–2141.

1973. Underwater observations on tracks of dredges and trawls and some effects of dredging on a scallop ground. J. Fish. Res. Board Can. 30: 173–180.

Dickie, L. M. 1955. Fluctuations in abundance of the giant scallop, *Placopecten magellanicus* (Gmelin), in the Digby area of the Bay of Fundy. J. Fish. Res. Board Can. 12: 797–856.

Dickie, L. M., and J. C. Medcof. 1963. Causes of mass mortalities of scallops (*Placopecten magellanicus*) in the southwestern Gulf of St. Lawrence. J. Fish. Res. Board Can. 20: 451–482.

Elner, R. W. 1978. The mechanics of predation by the shore crab, *Carcinus maenas* (L.), on the edible mussel *Mytilus edulis* L. Oecologia (Berlin) 36: 333–344.

Elner, R. W., and R. N. Hughes. 1978. Energy maximization in the diet of the shore crab, *Carcinus maenas*. J. Anim. Ecol. 47: 103–116.

Ennis, G. P. 1973. Food, feeding, and condition of lobsters, *Homarus americanus*, throughout the seasonal cycle in Bonavista Bay, Newfoundland. J. Fish. Res. Board Can. 30: 1905–1909.

Evans, P. D., and K. H. Mann. 1977. Selection of prey by lobster (*Homarus americanus*), when offered a choice between sea urchins and crabs. J. Fish. Res. Board Can. 34: 2203–2207.

Hirtle, R. W. M., and K. H. Mann. 1978. Distance chemoreception and vision in the selection of prey by American lobster (*Homarus americanus*). J. Fish. Res. Board Can. 35: 1006–1008.

Krantz, G. E., and J. V. Chamberlin. 1978. Blue crab predation on cultchless oyster spat. Proc. Nat. Shellfish. Assoc. 68: 38–41.

Mann, K. H. 1977. Destruction of kelp beds by sea urchins: a cyclical phenomenon or irreversible degradation? Helgol. Wiss. Meeresunters. 30: 455–467.

Mann, K. H., and P. A. Breen. 1972. The relation between lobster abundance, sea urchins and kelp beds. J. Fish. Res. Board Can. 29: 603–605.

Medcof, J. C., and N. Bourne. 1964. Causes of mortality of the sea scallop *Placopecten magellanicus*. Proc. Nat. Shellfish. Assoc. 53: 35–50.

Merrill, A. S., and J. A. Posgay. 1964. Estimating the natural mortality rate of the sea scallop, *Placopecten magellanicus*. Int. Comm. Northwest Atl. Fish. Res. Bull. 1: 88–107.

Scarratt, D. J. 1975. Observations on lobsters and scallops near Pictou, N.S. Fish. Mar. Serv. Res. Dev. Tech. Rep. 532: 6 p.

Scarratt, D. J., and R. Lowe. 1972. Biology of rock crab (*Cancer irroratus*) in Northumberland Strait. J. Fish. Res. Board Can. 29: 161–166.

Squires, H. J. 1970. Lobster (*Homarus americanus*) fishery and ecology in Port au Port Bay, Newfoundland, 1960–65. Proc. Nat. Shellfish. Assoc. 60: 22–39.

Weiss, H. M. 1970. The diet and feeding behaviour of the lobster, *Homarus americanus*, in Long Island Sound. Ph.D. Thesis, University of Connecticut, Storrs, Conn.

Whetstone, J. M., and A. G. Eversole. 1978. Predation on hard clams, *Mercenaria mercenaria*, by mud crabs, *Panopeus herbstii*. Proc. Nat. Shellfish. Assoc. 68: 42–48.

Possible Adaptive Significance of Certain Enzyme Polymorphisms in Steelhead Trout (*Salmo gairdneri*)[1]

J. M. REDDING AND C. B. SCHRECK

Oregon Cooperative Fishery Research Unit,[2] Oregon State University, Corvallis, OR 97331, USA

REDDING, J. M., AND C. B. SCHRECK. 1979. Possible adaptive significance of certain enzyme polymorphisms in steelhead trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 36: 544–551.

In experimentally reared winter steelhead trout (*Salmo gairdneri*) fry, mean weights, lengths, and condition factors of three isozyme phenotypes of lactate dehydrogenase (LDH) enzyme differed significantly. Time of emergence from the gravel was unrelated to LDH phenotype. Relative mortality of the phenotypes between eyed-egg stage and emergence was unaffected by different subgravel conditions of temperature and dissolved oxygen. Differential tolerance to acute challenges of high temperature and low dissolved oxygen was observed between phenotypes of isocitrate dehydrogenase (IDH) enzyme and LDH in juvenile trout. Parental effects may have biased the result for LDH. Differences between IDH phenotypes may be related to intrinsic properties of variant isozymes.

Key words: isozymes, lactate dehydrogenase, isocitrate dehydrogenase, temperature, dissolved oxygen, adaptive significance, *Salmo gairdneri*

REDDING, J. M., AND C. B. SCHRECK. 1979. Possible adaptive significance of certain enzyme polymorphisms in steelhead trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 36: 544–551.

Chez des alevins de truite steelhead d'hiver (*Salmo gairdneri*) élevés expérimentalement, on observe des différences significatives dans le poids, la longueur et les facteurs de condition moyens de trois phénotypes d'isozymes de l'enzyme lactate déshydrogénase (LDH). Le moment de l'émergence des alevins du gravier n'a pas de rapport avec le phénotype de LDH. La mortalité relative des phénotypes entre le stage d'oeuf oculé et l'émergence n'est pas affectée par des conditions différentes de température et d'oxygène dissous sous la surface du gravier. Nous observons des différences dans la tolérance à des expositions intenses à une température élevée et un bas niveau d'oxygène dissous entre phénotypes de l'enzyme isocitrate déshydrogénase (IDH) et LDH chez de jeunes truites. Les résultats obtenus avec LDH peuvent être biaisés par des effets parentaux. Il se peut que les différences entre les phénotypes d'IDH soient reliés à des propriétés intrinsèques d'isozymes différentes.

Received September 13, 1978
Accepted January 29, 1979

Reçu le 13 septembre 1978
Accepté le 29 janvier 1979

STOCKS of steelhead trout (*Salmo gairdneri*) in the Pacific Northwest exhibit a conspicuous dichotomy in the frequencies of alleles that code for polymorphisms of lactate dehydrogenase (LDH) and isocitrate dehydrogenase (IDH) (Allendorf 1975). Stocks inhabiting coastal areas differ from stocks that originate in interior regions of the Columbia and Snake River systems. In this study we attempt to relate these and other enzyme polymorphisms (isozymes) to their possible functional and adaptive significance.

LDH catalyzes the interconversion of pyruvate and lactate and regulates the balance between aerobic respiration and anaerobic glycolysis. Many species of fish possess LDH polymorphisms (Markert and Faulhaber 1965) and the molecular and genetic bases of these isozymes are well understood (Markert 1968). Internal and external factors can modulate the relative activity of LDH isozymes. Temperature-dependent induction of LDH isozymes occurs during thermal acclimation in a diverse array of vertebrate poikilotherms (Aleksiuk 1971; Hochachka 1965; Kunnemann 1973; Tsugawa 1976; Tsukuda 1975; Hochachka and Somero 1968). Photoperiod may affect LDH isozyme activity in fish (Kent and Hart 1976). Kinetic differences between LDH isozymes in temperature optima and substrate affinities exist in the fathead minnow, *Pimephales promelas* (Merritt 1972), sockeye salmon, *Oncorhynchus nerka* (Utter et al. 1974), and rainbow trout, *Salmo gairdneri* (Somero and Hochachka 1969; Hochachka and Somero 1968). Tsuyuki and Williscroft (1973) noted differences in the pH optima of LDH isozymes in rainbow trout and later (1977) demonstrated that swimming endurance was related to LDH phenotype. Geographic variation in the frequencies

[1]Oregon State University Agricultural Experiment Station Technical Paper No. 4887.

[2]Cooperators are Oregon Department of Fish and Wildlife, Oregon State University, and U.S. Fish and Wildlife Service.

Printed in Canada (J5375)
Imprimé au Canada (J5375)

of alleles that code for LDH polymorphisms has been correlated with environmental temperature (Merritt 1972; Mitton and Koehn 1975). Johnson (1971) demonstrated differential thermal tolerance between LDH phenotypes for a species that exhibits a latitudinal cline in the frequencies of LDH alleles.

Similar observations have been recorded for other polymorphic enzymes in trout: IDH (Moon and Hochachka 1972), citrate synthase (Hochachka and Lewis 1970), acetylcholinesterase (Baldwin and Hochachka 1970), and alkaline phosphatase (Whitmore and Goldberg 1972).

The purpose of this study was to investigate the adaptive significance of various enzyme polymorphisms, particularly those of LDH, in steelhead trout in relation to environmental temperature and dissolved oxygen. Specifically, we tested the hypotheses that (1) isozyme phenotypes of LDH in steelhead trout have differential survival and growth performances during the period between the eyed-egg stage and emergence, when exposed to different combinations of subgravel temperature and dissolved oxygen; and (2) in juvenile steelhead trout, isozyme phenotypes of LDH, IDH, and several other enzyme systems possess different tolerances to acute high temperature and low dissolved oxygen challenges.

Materials and Methods

The North Santiam River (Oregon) winter steelhead trout stock possesses three electrophoretic variants of LDH. Following the nomenclature of Wright et al. (1975) we designated these variants as the B′B′, B′B, and BB phenotypes of the B_1 locus in both liver and eye tissue. These phenotypes are known to exhibit simple Mendelian inheritance patterns (Utter et al. 1973). Because we wanted to test the relative performances of the three phenotypes, it was necessary that they have approximately equal representation in the test groups. Since the phenotypes are not present in equal proportions in the wild, the experimental fish were selectively bred.

Steelhead trout in reproductive condition were captured during their upstream migration in a trap at Minto Pond on the North Santiam River on May 7, 1976. The sperm or eggs from six males and six females were held on ice while the LDH phenotype was determined for each fish by electrophoretic analysis of liver tissue homogenates. Gametes were then selectively crossed so that a single male fertilized the eggs of a single female. The six families resulting from these crosses were mixed randomly to produce an experimental population. The fertilized eggs were held in Heath® incubators until they reached the eyed-egg stage; they were then shocked to identify viable eggs, and divided into two groups. One group was used for the subgravel incubation experiment. The other group was kept in the incubators until the fish absorbed their yolk sacs; then they were reared in circular tanks until used in the acute challenge experiments. Various physiochemical conditions in the circular tanks were held constant: temperature, 12°C; hardness ($CaCO_3$), 99 mg/L; pH, 7.3; conductivity, 241 μmho; dissolved oxygen, 8–12 mg/L.

Electrophoretic analyses were conducted according to the methods described by Utter et al. (1974). LDH phenotype was determined from eye tissue homogenates for trout fry and from liver tissue homogenates for juveniles. In addition, phenotype were determined for four enzyme systems in juvenile trout: IDH and tetrazolium oxidase (TO) in liver tissue and malate dehydrogenase (MDH) and alpha-glycerophosphate dehydrogenase (AGPDH) in white muscle tissue. Phenotype designations for these enzyme systems followed that of Allendorf (1973) and Utter et al. (1973). We recognized six phenotypes for IDH (AA, A^1A, A^1A^1, AA^3, A^1A^3, A^3A^3) and three each for TO (AA, AB, BB), MDH (B′B′, B′B, BB), and AGPDH (AA, AB, BB).

Experimental Design and Results

Subgravel Incubation Experiment

Twenty-two days after fertilization, 400 viable eyed eggs from the experimental population were buried beneath 25 cm of gravel (≥1.27 cm diam) in each of 12 flow-through incubation boxes (23 × 23 × 61 cm). The eggs were subjected to one of four treatment regimes of temperature and dissolved oxygen (DO). Each treatment had three replicates. Roman numerals indicate treatment designations:

Temp (°C)	Dissolved oxygen (mg/L) 5	9–10
12	III	IV
16	I	II

Dissolved oxygen was maintained at a constant concentration by a system in which nitrogen gas was diffused through inflowing water (Eddy 1971). Water velocity through the boxes was constant at 50 cm/h. Emergent fry that had completely absorbed their yolk sacs were collected daily. Individual fish were weighed and measured immediately after collection and then frozen for 1–4 wk before electrophoretic analysis.

Treatment effects produced no significant differences in the relative proportion of surviving fish for each LDH phenotype (Table 1). Confidence intervals ($P < 0.05$) were calculated for the observed proportions (p) by the formula $p\sqrt{1.96\ p(1\text{-}p)/n}$, where n equaled the sample

TABLE 1. Percentage of three phenotypes of LDH and the frequency of the *B* allele for emergent steelhead trout fry and a control sample from a circular tank (± = 95% confidence interval).

Treatment	Temp. (°C)	Dissolved oxygen (mg/L)	No. of fry	Percentage of phenotype BB	B′B	B′B′	Frequency of *B* allele
I	16	5	660	28	28	44	0.42±0.03
II	16	9–10	258	31	31	38	0.47±0.04
III	12	5	587	30	30	40	0.45±0.03
IV	12	9–10	627	33	25	42	0.46±0.03
Control	12	8	174	25	27	48	0.39±0.05

size. Because the number of eggs contributed by each female varied, the exact proportion of each phenotype was unknown before the eggs were buried; thus, we could not estimate the relative survival rates between phenotypes within a single treatment. There were no significant differences between the replicates within a treatment group.

Mean time of emergence, wet weight (W), fork length (L), and condition factor (W/L^3) for each LDH phenotype, replicate, and treatment were analyzed with a three-way analysis of variance (ANOVA) of means (Snedecor and Cochran 1967). There were no significant or consistent differences between the mean times of emergence for LDH phenotypes within any treatment group. Significant differences in time of emergence were observed between treatment groups as one would expect on the basis of bioenergetic considerations (Warren 1971).

For all treatment groups, fish of the BB phenotype of LDH weighed more than the B′B′ phenotype. Both homozygous forms weighed more than the heterozygous form, B′B (Fig. 1). The ANOVA for mean weight of emergent fry showed highly significant differences for both treatments and LDH phenotypes. Replications within treatments were homogenous. For a given phenotype, the fry reared at 12°C, 9–10 mg/L DO tended to weigh more at emergence than did the fry in either of the 16°C groups. The differences between treatment groups are consistent with the findings of others (Warren 1971).

Differences in length and condition factor between LDH phenotypes were highly significant. Differences between treatments were significant ($P < 0.10$). Replications within treatments were statistically homogenous. The BB phenotype had the greatest mean length and condition factor in all treatments (Fig. 1). B′B was the shortest and had the lowest condition factor, except in the 16°C, 5 mg/L DO treatment group in which the B′B′ form had the lowest condition factor.

Differences in weight, length, and condition factors between LDH phenotypes that were apparent at emergence were not observed 9 mo after fertilization in a sample of siblings from the same experimental population that were reared in incubation trays and circular tanks at 12°C, 9–10 mg/L DO.

A separate sample of 250 eyed eggs was taken on June 6, 1977 from North Santiam winter steelhead trout reared at Marion Forks Hatchery, Oregon. These fish were held in incubators until they had completely absorbed their yolk sacs. The fry were then weighed, measured, and analyzed for LDH phenotype. There were no significant differences in the mean weights, lengths, or condition factors between LDH phenotypes in the hatchery-reared fish.

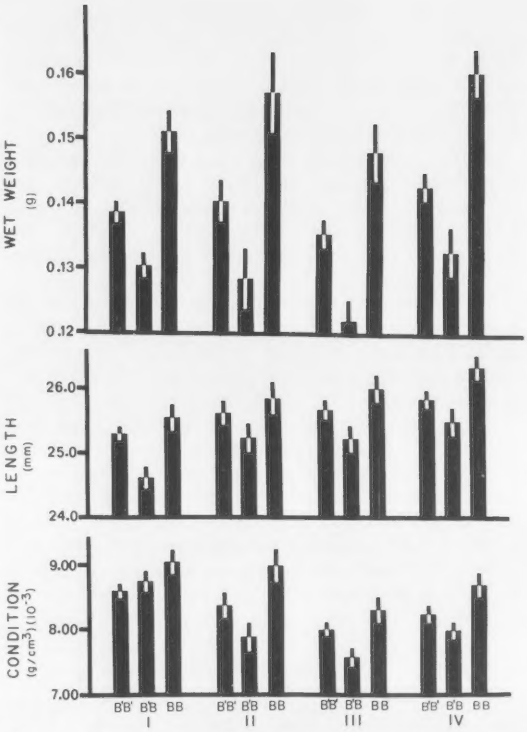


FIG. 1. Mean wet weights, length, and condition factors and confidence intervals ($P < 0.05$; vertical bars) of the three phenotypes of LDH in steelhead trout for each treatment, after incubation under gravel at various thermal and dissolved oxygen regimes: I = 16°C, 5 mg/L DO; II = 16°C, 9–10 mg/L DO; III = 12°C, 5 mg/L DO; IV = 12°C, 9–10 mg/L DO.

ACUTE CHALLENGE EXPERIMENTS

For the acute challenge experiments, groups of 100 juvenile trout (5–10 cm long) were placed in identical 125-L tanks and allowed to acclimate for 7–9 d before challenged. Fish were not fed the day before the start of the challenge. Immediately after death, each fish was weighed, measured, and frozen. Later, the fish were analyzed for LDH, IDH, AGPDH, MDH, and TO phenotypes. Time of death for individual fish was calculated from the time at which the ambient acclimation conditions were altered. We used a probit analysis adapted from Bliss (1938) to calculate the median survival time (MST) and its confidence interval ($P < 0.05$) for each phenotype.

TEMPERATURE

In the acute high temperature challenge experiment three replicate groups from the experimental population were acclimated at 12°C and then subjected to a rapid increase in water temperature. After 375 min, water temperature had reached 26.5°C; it was main-

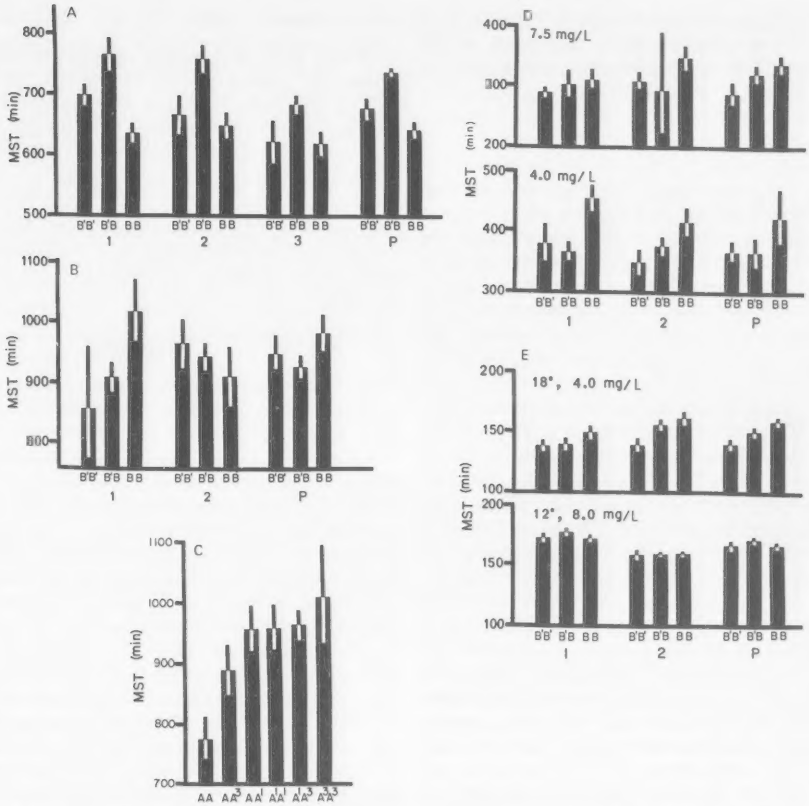


FIG. 2. Median survival times (MST) and confidence intervals ($P < 0.05$; vertical bars) of (A) the three phenotypes of LDH for the acute high temperature challenge experiment with juvenile trout from the experimental population (three replicate samples and a pooled sample (P) are shown, fish acclimated at 12°C); (B) the three phenotypes of LDH for the acute high temperature challenge experiment with hatchery-reared juvenile trout (two replicate samples and a pooled sample are shown, fish acclimated at 12°C); (C) the six phenotypes of IDH from the acute high temperature challenge experiment with hatchery-reared juvenile trout (data are pooled from two replicate samples, fish acclimated at 12°C); (D) the three phenotypes of LDH for the acute low dissolved oxygen challenge experiment with juvenile trout from the experimental population (two replicate samples and a pooled sample are shown for each of two acclimation regimes, 7.5 mg/L and 4.0 mg/L dissolved oxygen); and (E) the three phenotypes of LDH for the acute simultaneous high temperature and low dissolved oxygen challenge experiment with juvenile trout from the experimental population (two replicate samples and a pooled sample are shown for each of two acclimation regimes, 18°C, 4.0 mg/L and 12°C, 8.0 mg/L dissolved oxygen).

tained at this level for the duration of the experiment. All fish died within 1230 min.

Two groups of 100 juvenile trout reared at Marion Forks Hatchery and randomly bred from the same stock and brood year as the experimental population were acclimated at 12°C and then exposed to a challenge of 26.0°C. The maximum temperature was reached in 300 min. All fish died within 1860 min. Only the liver tissue enzymes, LDH, IDH, and TO. were assayed for the hatchery-reared groups.

In the experimental population the B′B phenotype of LDH exhibited the highest MST, and BB had the lowest (Fig. 2A). In the pooled sample, confidence intervals for the MST's of LDH phenotypes do not overlap. The MST data from the hatchery-reared fish showed no consistent differences between LDH phenotypes (Fig. 2B).

There was differential tolerance to acute high temperature challenge by the phenotypes of IDH for both the experimental population and the hatchery-reared

fish. In the experimental population the A^3A^3 phenotype tended to have the highest MST. Similarly, in the hatchery fish, A^3A^3 was significantly more tolerant than either AA or AA^3 (Fig. 2C).

Dissolved Oxygen

Two replicate groups each from the experimental population were acclimated to 7.5 and 4.0 mg/L DO at 12°C. The challenge condition of 1.5 mg/L DO was reached within 270 min and sustained for the duration of the experiment. All fish that were acclimated at 7.5 mg/L DO died within 675 min. About 10% of the fish that were acclimated at 4.0 mg/L DO survived until the experiment was terminated after 2880 min. The relationships of probits vs. log time were curvilinear for the groups in which mortality was incomplete; therefore, we used data only from the linear portion of the curves in the probit analyses for these groups.

For both acclimation groups, the BB phenotype of LDH was more tolerant than the B′B′ phenotype (Fig. 2D). In the pooled samples the B′B phenotype was intermediate in tolerance for the 7.5 mg/L DO group but equivalent to the B′B′ in the 4.0 mg/L DO acclimation group.

The IDH phenotypes had differential tolerance to low dissolved oxygen challenge. In general, the A^1A^1 phenotype had a greater MST than any of the other five phenotypes. Of the 24 fish that survived the duration of the experiment, 17 were A^1A^1, a far greater number than one would expect if differential tolerance did not exist.

Temperature and Dissolved Oxygen

Acute challenges of high temperature and low dissolved oxygen were administered simultaneously. Two replicate groups from the experimental population were tested for each of two acclimation regimes. Fish were acclimated at either 12°C, 8.0 mg/L DO or 18°C, 4.0 mg/L DO. The fish were subjected to rapidly increasing temperature (2–3°C/h) and decreasing dissolved oxygen (2.5–3.5 mg/L/h). All fish died within 225 min, and before the target conditions of 26.5°C, 1.5 mg/L DO were reached.

For fish acclimated at 18°C, 4.0 mg/L DO, the BB phenotype of LDH was significantly more tolerant than B′B′. The MST value for B′B was intermediate (Fig. 2E). There were no significant differences between the MST's of the three LDH phenotypes for the groups acclimated at 12°C, 8.0 mg/L DO.

Differential tolerance was apparent between the IDH phenotypes for those fish acclimated at 18°C, 4.0 mg/L DO. As in the acute low dissolved oxygen experiment, the A^1A^1 phenotype tended to have a higher MST than did the other IDH phenotypes. There were no obvious differences between phenotypes of IDH for groups acclimated at 12°C, 8.0 mg/L DO.

Other Observations

There were no apparent differences in tolerance between the phenotypes of TO, MDH, or AGPDH for any of the acute challenge experiments. No significant correlation existed between the weight or condition factor of individual fish and their time to death in any of the acute challenge experiments.

Separate groups of 100 fish from the experimental population were held for 9 d in 125-L tanks under conditions that approximated each acclimation regime of each acute challenge experiment. None of the control fish died.

Discussion

Isozyme phenotypes in the experimental population of steelhead trout differed in weight, length, and condition factor of emergent fry (LDH only) and in the tolerance of juvenile trout to acute challenges of high temperature and low dissolved oxygen (LDH and IDH). Time of emergence from the gravel was not related to LDH phenotype. Differences in subgravel temperature and dissolved oxygen had no significant effect on the proportionate survival of LDH phenotypes.

We assume that the isozyme phenotypes observed in this study are controlled exclusively by the genome of the fish and are not affected qualitatively by environmental factors. That these isozyme systems exhibit predictable Mendelian inheritance patterns is taken as evidence of the validity of this assumption. Furthermore, the environmental induction of enzymes is believed to require a period of 1–2 wk (Hazel and Prosser 1974), making it an unlikely event during a 48-h bioassay.

Granting the above assumption, two factors, singly or in combination, may be responsible for the differences we observed. First, they may be related intrinsically to the enzyme polymorphisms. In other words, the functional properties of variant isozymes may result in differential performances by the fish. The characteristics we observed (i.e. growth and tolerance) are undoubtedly influenced by many gene–enzyme systems; thus, it seems unlikely that allelic combinations at a single locus could be entirely responsible for the observed effects. At best, our results provide correlations that may suggest causal relationships between isozyme variants and performance. We do not exclude the possibility of epistatic interaction with other genes. Secondly, the results may be biased by uncontrolled genetic variability between family groups (parental effects). The experimental population was derived from only six families; therefore, the probability that parental effects influenced the parametric means is high. Because we could not identify the parentage of individual fish, comparisons between family groups were impossible.

Comparable results from fish that were the progeny of a large number of random matings, such as one would expect to find in a hatchery, would support the hypothesis of intrinsic enzyme-related differences. Pre-

sumably, under such conditions the probability that a single mating would significantly influence the parametric means of the filial population is small.

Steelhead trout fry of the 1977 year-class that were spawned and reared at Marion Forks Hatchery until the eyed-egg stage did not differ in length, weight, or condition factor between LDH phenotypes. This fact implicates parental effects as the most likely cause of the size differences observed between LDH phenotypes in the experimental population. However, it is also possible that environmental factors (e.g. considerably lower water temperature) at Marion Forks Hatchery during early embryonic development of the fish effectively altered or masked differences that might be manifest under other conditions.

Independent results on the growth of different LDH phenotypes for Deschutes River steelhead trout provide supportive evidence for intrinsic LDH-related size differentiation. Reisenbichler and McIntyre (1976) used LDH as a genetic marker to distinguish the progeny of multiple matings of hatchery and wild trout. Their experimental crosses were designed specifically to maximize non-LDH genetic variability (i.e. minimize parental effects). In all groups the BB phenotype was largest at the eyed-egg stage ($P < 0.01$) (R. R. Reisenbichler, U.S. Fish and Wildlife Service, Red Bluff, Calif., personal communication). The B′B′ phenotype had the smallest eggs while B′B had eggs of intermediate size. As in the eyed-egg stage, swim-up fry of the BB phenotype had the largest volume. However, B′B had a smaller volume than B′B′, contrary to what one might expect from the relative sizes of the eggs. This observation (i.e. BB > B′B′ > B′B) parallels our findings for the experimental population of North Santiam fry (Fig. 1). Furthermore, R. R. Reisenbichler's unpublished data show that in the wild environment there were no significant differences or consistent trends between the mean lengths of the LDH phenotypes 4 mo after fertilization, a condition that is analogous to the lack of LDH-related size differentiation in juveniles of the experimental population.

Because of the pivotal nature of LDH with respect to anaerobic glycolysis in the white muscle and gluconeogenesis in the liver, it is conceivable that functional differences between isozyme variants of LDH could translate into differences in growth performance. Functional differences between LDH isozymes are known to occur in rainbow trout (Tsuyuki and Williscroft 1973; Hochachka and Somero 1968). Tsuyuki and Williscroft (1977) correlated variation in swimming stamina in rainbow trout to LDH phenotype. Our results generally support the contention that LDH polymorphism in trout, under some conditions, has some adaptive significance with respect to body size at the time of emergence from the gravel.

The geographic distribution of alleles coding for particular isozymes sometimes provides an indication of the adaptive significance of those isozymes (Johnson 1971; Koehn 1970). Stocks of steelhead trout in the Pacific Northwest exhibit a conspicuous dichotomy in the frequencies of the two alleles that code for the LDH polymorphisms described here. Coastal stocks have a predominance of the *B* allele while interior stocks of the Columbia and Snake River systems possess higher frequencies of the alternate *B′* allele (Allendorf 1975). Perhaps the size differentiation that we have observed between LDH phenotypes in trout fry is related to this phenomenon.

In the experimental population, the B′B phenotype of LDH exhibited a significantly greater tolerance to acute high temperature challenge than did either of the homozygous forms (Fig. 2A). Oddly, it was the BB phenotype, not B′B, that was most tolerant when subjected to low dissolved oxygen or simultaneous low dissolved oxygen and high temperature (Fig. 2D, E). In nature, increased water temperature is usually concomitant with decreased dissolved oxygen concentration. One would expect those fish that are more tolerant to high temperature also to be more tolerant to low dissolved oxygen. The absence of this relation in our experiments suggests that certain allelic combinations are better suited for either high temperature or low dissolved oxygen, but not both.

Again, these results could signify intrinsic differences between variant forms of LDH or they could simply indicate parental bias. Because hatchery-reared steelhead trout did not exhibit any consistent differential tolerance to high temperature with respect to LDH (Fig. 2B), we believe that simple parental effects are the most parsimonious explanation for the differences in thermal tolerance observed in the experimental population.

Although these results do not support the notion that variants of LDH are associated with differential tolerance, they do suggest that tolerance to high temperature or low dissolved oxygen is at least partially heritable. Significant differences in a trait between families are expected in this case only if the trait is heritable. The fact that tolerance to high temperature and low dissolved oxygen seems to be heritable could be useful to fishery managers who wish to manipulate stocks of steelhead trout for use in warmer or less aerobic systems. It also implies that alterations in the temperature or dissolved oxygen regimes of an aquatic system can affect the genetic constitution of steelhead trout populations.

Unlike the LDH system, differential tolerance to high temperature and low dissolved oxygen between isozyme phenotypes of IDH cannot be explained solely on the basis of parental effects. For the experimental population A^3A^3 tended to be most tolerant to high temperature relative to other IDH phenotypes. The A^3A^3 fish were produced in approximately equal numbers by five of the six experimental families. Thus, if parental effects were the only influence on performance one would expect a more random distribution than that which we

observed. Analogously, within the hatchery population the A^3A^3 phenotype showed the greatest tolerance to thermal challenge, significantly greater than that of the AA or AA^3 forms (Fig. 2C). Of the 24 fish that survived the low dissolved oxygen challenge 71% were A^1A^1. Differential tolerance of IDH phenotypes must be attributed, at least partially, to some intrinsic differences correlated to IDH polymorphisms.

The IDH enzyme catalyzes the reversible oxidation–decarboxylation of isocitrate and is the rate limiting enzyme in the Krebs cycle. Moon and Hochachka (1971, 1972) demonstrated differential temperature dependency for the reaction kinetics of IDH isozymes in rainbow trout. Our results corroborate their interpretation that IDH isozymes are of adaptive significance in this species.

Interior stocks from the Snake and Deschutes River systems tend to have higher frequencies of the A^1 and A^3 alleles of IDH than do coastal stocks of steelhead trout (Oregon Cooperative Fishery Research Unit unpublished data). Our experiments suggest that the isozyme phenotypes of the A^1 and A^3 alleles may possess a greater tolerance to high temperature than those of the A allele (Fig. 2C). Water temperature in the Snake River may rise as high as 25°C in the summer (Beiningen and Ebel 1971), a value that approaches the upper thermal tolerance limit of steelhead trout. In contrast, a typical coastal stream in Oregon may have summer thermal maximums of only 15°C (Moring 1975). If water temperature during the summer is higher in the interior regions than in coastal areas, a possible consequence might be the natural selection of isozyme phenotypes with greater tolerance to high temperature in interior stocks. The relatively higher frequencies of A^1 and A^3 alleles in interior stocks is consistent with this explanation.

Our results support the notion that enzyme polymorphisms within fish species can have adaptive significance to the organism. This contrasts to the opposing view (e.g. King and Jukes 1969) that most protein variation is effectively neutral and that protein evolution occurs via random fixation processes rather than by natural selection. Polymorphisms of LDH within steelhead trout populations appear to be correlated in some cases to size differences between individual fry. Polymorphisms of IDH, but not LDH, seem to be associated with differential tolerance to acute challenges of high temperature and low dissolved oxygen.

Acknowledgments

We appreciate the contributions of Drs J. D. McIntyre and N. Hartman with respect to the design and statistical analysis of our study. R. R. Reisenbichler provided unpublished data.

ALEKSIUK, M. 1971. An isoenzyme basis for instantaneous cold compensation in reptiles: Lactate dehydrogenase kinetics in *Thamnophis sirtalis*. Comp. Biochem. Physiol. 40B: 671–681.

ALLENDORF, F. W. 1973. Genetic variation, inheritance and preliminary population distribution of some proteins of *Salmo gairdneri*. M.S. Thesis, Univ. of Washington, Seattle, Wash. 61 p.

1975. Genetic variability in a species possessing extensive gene duplication: Genetic interpretation of duplicated loci and examination of genetic variation in populations of rainbow trout. Ph.D. Thesis, Univ. of Washington, Seattle, Wash. 98 p.

BALDWIN, J., AND P. W. HOCHACHKA. 1970. Functional significance of isoenzymes in thermal acclimatization: Acetylcholinesterase from trout brain. Biochem. J. 116: 883–887.

BEININGEN, K., AND W. EBEL. 1971. Dissolved nitrogen, dissolved oxygen, and related water temperatures in the Columbia and lower Snake Rivers, 1965–1969. U.S. Nat. Mar. Fish. Serv. Data Rep. 56: 60 p.

BLISS, C. I. 1938. The determination of dosage mortality curves from small numbers. Q. J. Pharm. Pharmacol. 11: 192–216.

EDDY, R. M. 1971. The influence of dissolved oxygen concentration and temperature on the survival and growth of chinook salmon embryos and fry. M.S. Thesis, Oregon State Univ., Corvallis, Oreg. 45 p.

HAZEL, J. R., AND C. L. PROSSER. 1974. Molecular mechanisms of temperature compensation in poikilotherms. Physiol. Rev. 54: 620–677.

HOCHACHKA, P. W. 1965. Isoenzymes in metabolic adaptation of a poikilotherm: Subunit relationships in lactate dehydrogenases of goldfish. Arch. Biochem. Biophys. 111: 96–103.

HOCHACHKA, P. W., AND J. K. LEWIS. 1970. Enzyme variants in thermal acclimation: Trout liver citrate synthases. J. Biol. Chem. 245: 6567–6573.

HOCHACHKA, P. W., AND G. N. SOMERO. 1968. The adaptation of enzymes to temperature. Comp. Biochem. Physiol. 27: 659–668.

JOHNSON, M. S. 1971. Adaptive lactate dehydrogenase variation in the crested blenny, *Anoplarcus*. Heredity 27: 205–226.

KENT, J. D., AND R. G. HART. 1976. The effect of temperature and photoperiod on isozyme induction in selected tissues of the creek chub, *Semotilus atromaculatus*. Comp. Biochem. Physiol. 54B: 77–80.

KING, J. L., AND T. H. JUKES. 1969. Non-Darwinian evolution. Science 164: 788–798.

KOEHN, R. K. 1970. Functional and evolutionary dynamics of polymorphic esterases in catostomid fishes. Trans. Am. Fish. Soc. 99: 219–223.

KUNNEMANN, H. 1973. The influence of temperature on thermostability, isoenzyme pattern and reaction kinetics of lactate dehydrogenase from fishes. Mar. Biol. 18: 37–45.

MARKERT, C. L. 1968. The molecular basis for isozymes. Ann. N.Y. Acad. Sci. 151: 14–40.

MARKERT, C. L., AND I. FAULHABER. 1965. Lactate dehydrogenase isozyme patterns of fish. J. Exp. Zool. 159: 319–332.

MERRITT, R. B. 1972. Geographic distribution and enzymatic properties of lactate dehydrogenase allozymes in the fathead minnow, *Pimephales promelas*. Am. Nat. 106: 173–184.

MITTON, J. B., AND R. K. KOEHN. 1975. Genetic organization and adaptive response of allozymes to ecological variables in *Fundulus heteroclitus*. Genetics 79: 97–111.

MOON, T. W., AND P. W. HOCHACHKA. 1971. Temperature and enzyme activity in poikilotherms: Isocitrate dehydro-

genases in rainbow trout liver. Biochem. J. 123: 695–705.

1972. Temperature and the kinetic analysis of trout isocitrate dehydrogenases. Comp. Biochem. Physiol. 42B: 725–730.

MORING, J. R. 1975. The Alsea watershed study: Effects of logging on the aquatic resources of three headwater streams of the Alsea River, Oregon. Part II — Changes in environmental conditions. Oreg. Dep. Fish Wildl. Fish. Res. Rep. 9: 39 p.

REISENBICHLER, R. R., AND J. D. MCINTYRE. 1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, *Salmo gairdneri*. J. Fish. Res. Board Can. 34: 123–128.

SNEDECOR, G. W., AND W. G. COCHRAN. 1967. Statistical methods. Iowa State Univ. Press, Ames, Iowa 593 p.

SOMERO, G. N., AND P. W. HOCHACHKA. 1969. Isoenzymes and short-term temperature compensation in poikilotherms: Activation of lactate dehydrogenase isoenzymes by temperature decreases. Nature 223: 194–195.

TSUGAWA, K. 1976. Direct adaptation of cells to temperature: Similar changes of lactate dehydrogenase isozyme patterns by in vitro and in situ adaptations in *Xenopus laevis*. Comp. Biochem. Physiol. 55B: 259–263.

TSUKUDA, H. 1975. Temperature dependency of the relative activities of liver lactate dehydrogenase isozymes in goldfish acclimated to different temperatures. Comp. Biochem. Physiol. 52B: 343–345.

TSUYUKI, H., AND S. N. WILLISCROFT. 1973. The pH activity relations of two LDH homotetramers from trout liver and their physiological significance. J. Fish. Res. Board Can. 30: 1023–1026.

1977. Swimming stamina differences between genotypically distinct forms of rainbow (*Salmo gairdneri*) and steelhead trout. J. Fish. Res. Board Can. 34: 996–1003.

UTTER, F. M., H. O. HODGINS, AND F. W. ALLENDORF. 1974. Biochemical genetic studies of fishes: Potentialities and limitations, p. 213–238. *In* C. C. Malins and J. R. Sargent [ed.] Biochemical and biophysical perspectives in marine biology. Vol. I. Academic Press, New York, N.Y.

UTTER, F. M., H. O. HODGINS, F. W. ALLENDORF, A. G. JOHNSON, AND J. L. MIGHELL. 1973. Biochemical variants in Pacific salmon and rainbow trout: Their inheritance and application in population studies, p. 329–339. *In* J. H. Schroder [ed.] Genetics and mutagenesis of fish. Springer-Verlag, Berlin.

WARREN, C. E. 1971. Biology and water pollution control. W. B. Saunders, Philadelphia, Pa. 434 p.

WHITMORE, D. H., AND E. GOLDBERG. 1972. Trout intestinal alkaline phosphatases II. The effect of temperature on enzymatic activity in vitro and in vivo. J. Exp. Zool. 182: 59–68.

WRIGHT, J. E., F. R. HECKMAN, AND L. M. ATHERTON. 1975. Genetic and developmental analyses of LDH isozymes in trout, p. 375–403. *In* C. L. Markert [ed.] Isozymes. Vol. III. Academic Press, New York, N.Y.

Population-Genetic Study of the Effects of Selective Fishing on the Growth Rate of Trout

L. D. FAVRO AND P. K. KUO

Department of Physics, Wayne State University, Detroit, MI 48202, USA

AND J. F. MCDONALD

Department of Mathematics, University of Windsor, Windsor, Ont. N9B 3P4

FAVRO, L. D., P. K. KUO, AND J. F. MCDONALD. 1979. Population-genetic study of the effects of selective fishing on the growth rate of trout. J. Fish. Res. Board Can. 36: 552–561.

A mathematical model that simulates the effect of selective fishing on the size distributions of populations of trout is presented. The model assumes that the growth rate is genetically controlled by a small number of major genes. Empirical numbers for the growth and mortality rates of brown trout (*Salmo trutta*) in the Au Sable River of Michigan are used as input data. A computer is used to calculate the changes in the population subject to various degrees of fishing pressure for various size limits. Results are presented for periods of time ranging from 5–30 yr. The observed changes in the population over the period 1959 to 1977 in a test stretch of the Au Sable River agree quite well with the computations.

Keywords: fish, trout, genetics, growth, size limit, population, *Salmo trutta*

FAVRO, L. D., P. K. KUO, AND J. F. MCDONALD. 1979. Population-genetic study of the effects of selective fishing on the growth rate of trout. J. Fish. Res. Board Can. 36: 552–561.

Les auteurs présentent un modèle mathématique simulant l'effet d'une pêche sélective sur la répartition des tailles de populations de truites. Le modèle suppose que le rythme de croissance est réglé génétiquement par un petit nombre de gènes majeurs. Comme entrées, ils utilisent des chiffres empiriques se rapportant aux rythmes de croissance et de mortalité de la truite brune (*Salmo trutta*) dans la rivière Au Sable du Michigan. Ils utilisent un ordinateur pour calculer les changements qui se produisent dans la population sujette à diverses intensités de pêche et avec diverses limites de taille. On présente les résultats pour des périodes allant de 5 à 30 ans. Les changements observés dans la population durant la période 1959 à 1977 dans un segment expérimental de la rivière Au Sable s'accordent assez bien avec le modèle.

Received November 14, 1978
Accepted January 31, 1979

Reçu le 14 novembre 1978
Accepté le 31 janvier 1979

THE familiar anglers' lament that "the fishing isn't as good as it used to be" is usually regarded either as a failure of memory or a simple result of more complete cropping of the population because of increasing fishing pressure. It is much less often suggested that the decline in the number of larger fish caught by anglers might result from changes in the gene pool caused by selective fishing. This paper describes a mathematical model that has been developed to provide estimates of the magnitude of possible genetic effects resulting from long-standing fishing regulations that prescribe a minimum length (hereafter referred to as the "size limit") for the killing of captured fish. Such a limit produces, in effect, an unnatural selection process in which the faster growing fish are preferentially killed at an earlier age, perhaps even before they reach sexual maturity. In addition, even without a size limit, angling preferentially kills fish that grow faster because those fish require more food and therefore are more susceptible to the angler's lure. Recent experiments (e.g. Kincaid et al. 1977; Bridges 1973; Kincaid 1972; Aulstad et al. 1972; von Limbach 1970; Donaldson and Menasveta 1961) have shown that growth rate in salmonoids is strongly heritible. Thus selection on the basis of growth rate is expected to be reflected in the future population. Similar effects might be expected in commercial fisheries in which a minimum capture size has been determined by, for instance, the mesh diameter of nets.

While many species of fish are commonly subjected to selective fishing pressure we have focused our attention on trout, specifically the brown trout, *Salmo trutta*, and have used data from the Au Sable River system in Michigan to determine the parameters in our model. There are three reasons for this choice. First, large stretches of the Au Sable system have been subjected to intense fishing pressure with size limits in the range that might be expected to discriminate between fast and slow growers. Second, the Michigan Department of Natural Resources has a large amount of data, much

Printed in Canada (J5437)
Imprimé au Canada (J5437)

of them as yet uncompiled, on the population of *S. trutta* in these stretches of the Au Sable. These data have been collected over a period of nearly 20 yr by stream-shocking surveys, tagging studies, and by angler creel census. Third, in recent years there has been a dramatic decline in the percentage of larger fish found both in the angler catch and in stream-shocking samples in these stretches. While this decline is probably due to some complex combination of interacting factors, it seems desirable to obtain reasonable limits on the magnitude of the genetic contribution to the decline by computer simulation of that aspect of the problem. The model we use here is a conventional population-genetic model with a selection process (i.e. fishing pressure) that depends on the size of the individual fish.

Since the purpose of this study is to isolate and estimate the effects of selective fishing on the growth rate of trout, we first imagine a population that is not subject to other selective processes. The population is described by giving the number of fish of a given age of each genotype. A characteristic growth curve is assigned to each genotype. A computer is programmed to calculate the changes in the population numbers due to growth, mortality, and reproduction. The effect of selective fishing is simulated by letting those fish above the legal size limit have a higher mortality rate than those below the limit.

The organization of the computer program is outlined schematically in Fig. 1. The computer uses the growth curve of each genotype to calculate the increase in size of each group of fish during the year. Next the size information for each group is used to calculate the mortality incurred by the group for that year. These results are combined to predict the population numbers of each age and genetic group for the next spawning season. Standard genetic rules are then employed to calculate the addition to the population due to reproduction. The new population numbers are used to initiate the program for the ensuing year. Details of each block of the program are given in subsequent sections.

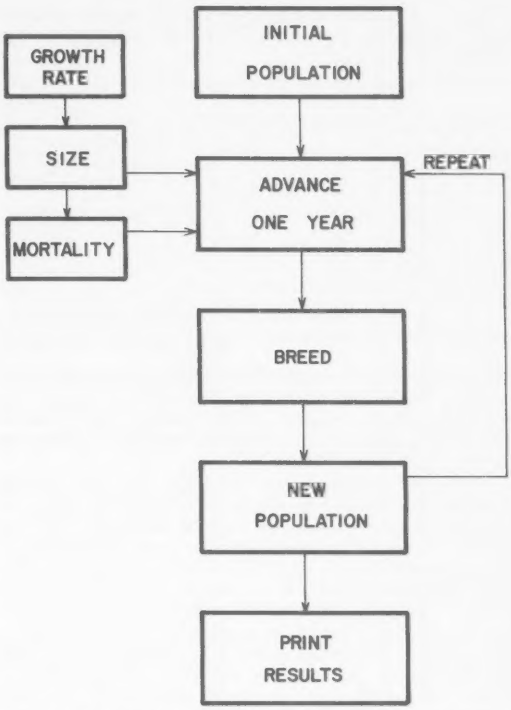


FIG. 1. Schematic diagram of the mathematical model.

Genetic Program

The construction of a completely realistic genetic model to describe the population dynamics of trout is complicated by a lack of detailed data about the process. However, there are some experimental results from Kincaid et al. (1977), which can serve as general guidelines for such a model. Using standard hatchery stocks, the authors have shown that when the fastest (or slowest) growing fish are bred together the offspring tend to be fast (slow) growers with a reduced spread in growth rates. This success with selective breeding would seem to indicate that the growth rate is principally controlled by a small number of major genes, perhaps as few as one or two. The fact that neither the fast × fast nor slow × slow crosses produce offspring with other than the parental growth rate indicates that for modeling purposes both groups can be considered to be homozygous with respect to this trait. Also, neither type seems to be dominant since crossing of fast and slow growing fish produced intermediate growth (H. L. Kincaid personal communication).

Fortunately, these few general experimental results are enough to indicate the overall behavior of the evolution of the size structure of a trout population under the selective pressures of angling. In other words, the population dynamics of genetic models are insensitive to many of the details of the genetic process provided the general features described above are included in the model. Accordingly, we have attempted to construct a model that is both faithful to these general genetic features and mathematically tractable without losing the possibility of future generalizations to more complicated situations.

The model assigns the growth rate to two unlinked loci with two alleles at each locus. One-locus results can be obtained by simply making one of the loci neutral with respect to a given trait or, alternatively, two different traits can be attributed to the loci without modifying the model. We will designate the alleles at one locus by a_1 and a_2 and the alleles at the other locus by b_1 and b_2. There are then nine distinct genotypes of the form $a_i a_j b_k b_l$ with appropriate values of the indices i, j, k, l. (Here the combinations $a_1a_2\,[b_1b_2]$ and $a_2a_1\,[b_2b_1]$ are considered to be indistinguishable.) To facilitate further discussions we shall introduce a single index μ (running from 1 to 9) in place of this set of indices. The relation between the indices is given in Table 1.

It is assumed that all fish mate for the 1st time in their third fall (i.e. in the fall before they reach a full 3 yr) and the usual random-mating hypothesis is used. Hence, all possible backcrosses with fish from previous generations occur. The result of

TABLE 1. The connection between the *ijkl* indices and the μ index designating the genotype, and the ultimate average genetic size, $S_\mu(\infty)$.

μ	i	j	k	l	$S_\mu(\infty)$ (cm)
1	1	1	1	1	30
2	1	1	2	1	35
3	1	2	1	1	40
4	1	1	2	2	45
5	2	2	1	1	50
6	1	2	2	1	55
7	1	2	2	2	60
8	2	2	2	1	65
9	2	2	2	2	70

this backcrossing is a (realistic) slowing down of the characteristic time for the occurrence of genetic changes. In addition, it is assumed that the fecundity of female fish is proportional to their weight, which in turn is assumed to be proportional to the cube of their length.

Within the random-mating hypothesis the mathematical technique for determining the genetic makeup of each new generation involves calculating weighted gamete distributions for the male and female fractions of the existing population. The mathematical notation that is used here to accomplish this differs somewhat from that in most texts on genetics. The present language is designed both to facilitate computer programming and to minimize the actual operations performed by the computer. The symbol σ is used to designate the age of a fish. Numbers of male and female fish of a given age and genotype are indicated by $m_\mu(\sigma)$ and $n_\mu(\sigma)$, respectively. The greek letters α, β, γ, and δ are used to designate the fractional number of the four possible types of male gametes according to the following prescription:

α = fractional number of gametes of type a_2b_2,
β = fractional number of gametes of type a_2b_1,
γ = fractional number of gametes of type a_1b_2,
δ = fractional number of gametes of type a_1b_1.

It should be noted that $\alpha + \beta + \gamma + \delta = 1$. The fractional numbers of female gametes are designated by the same symbols with primes.

The numbers $\alpha, \beta, \gamma, \delta$ occurring in a random-mating calculation (remembering our assumption that all fish breed first in the fall of their 3rd yr) may be written in terms of the numbers $m_\mu(\sigma)$ as

$$\alpha = [M_9 + (M_8 + M_7)/2 + M_6/4]/M,$$
$$\beta = [(M_8 + M_5)/2 + M_6/4 + M_5]/M,$$
$$\gamma = [(M_7 + M_2)/2 + M_6/4 + M_4]/M,$$
$$\delta = [M_1 + (M_2 + M_3)/2 + M_6/4]/M,$$

where $M_\mu = \sum_{\sigma=3}^{15} m_\mu(\sigma)$, and $M = \sum_{\mu=1}^{9} M_\mu$.

It should be noted that for programming purposes we have included fish up to and including age 15. In the actual calculations (and in real populations) the number of fish of this age or higher is negligibly small.

The distribution of female gametes is calculated similarly except that a weighting factor proportional to the weight of the fish (i.e. the length cubed) is introduced to take account of the greater fecundity of the larger females. The fecundity is introduced here as a function of the *specific* size of the fish, i.e. as a function of the size, $S_\mu(\sigma)$, divided by the ultimate genetic size, $S_\mu(\infty)$. This provides a simple mathematical means of emphasizing the fecundity of the older females without introducing an unnatural destabilization of the genetic distribution which would result from using a function in which the size was not normalized in this way. Thus, for the female gamete distribution we can obtain α', β', γ', and δ' using the identical formulae as for α, β, γ, and δ shown above, but with M_μ replaced by N_μ and M by N where

$$N_\mu = \sum_{\sigma=3}^{15} (S_\mu(\sigma)/S_\mu(\infty))^3 n_\mu(\sigma), \quad \text{and} \quad N = \sum_{\mu=1}^{9} N_\mu.$$

Designating the fractional number of offspring of genotype μ, by F_μ, we obtain

$$F_1 = \delta\delta'$$
$$F_2 = \delta\gamma' + \gamma\delta'$$
$$F_3 = \delta\beta' + \beta\delta'$$
$$F_4 = \gamma\gamma'$$
$$F_5 = \beta\beta'$$
$$F_6 = \alpha\delta' + \delta\alpha' + \beta\gamma' + \gamma\beta'$$
$$F_7 = \alpha\gamma' + \gamma\alpha'.$$
$$F_8 = \alpha\beta' + \beta\alpha'$$
$$F_9 = \alpha\alpha'.$$

The program adjusts the overall fecundity so that the total number of fish (offspring plus survivors of previous generations) immediately after the birth of the new generation does not vary from year to year. This fixes the size of the population and eliminates distortions of the age distribution associated with an increasing or decreasing population. Thus, any changes in the distribution of sizes of the fish results purely from genetic effects and not from instabilities in population number.

Growth Rate

To calculate the size distributions of the various genotypes in our model we must have some curve, or set of curves, which relate their sizes to age. Such curves must reflect the well known decline in growth rate with advancing age and also provide the variety of growth rates observed in younger fish. The size function, $S_\mu(\sigma)$, we use can be represented by the formula

$$S_\mu(\sigma) = C_\mu \ln [(1 + A)/(1 + Ae^{-\lambda\sigma})].$$

(The particular methematical form for this curve has no special significance. All that is important is the general shape of the growth curve.) The values of the parameters A and $\lambda (A = 66.7;\ \lambda = 0.7)$ have been chosen by fitting the data of Alexander and Ryckman (1976) and Gowing (1975) for younger fish and the data of Favro and McGlinn (unpublished data) for older fish. The genetic contribution to the growth rate is contained in the parameter C_μ which is related to the average ultimate size, $S_\mu(\infty)$, which can be attained by fish of genotype μ through the formula

$$C_\mu = S_\mu(\infty)/\ln (1 + A).$$

The values of C_μ are selected to give ultimate genetic sizes ranging from 30 to 70 cm as shown in Table 1. The present population appears not to contain fish with the potential to reach the upper end of this range, but on the basis of old angling journals they are presumed to have existed previously and hence are included in

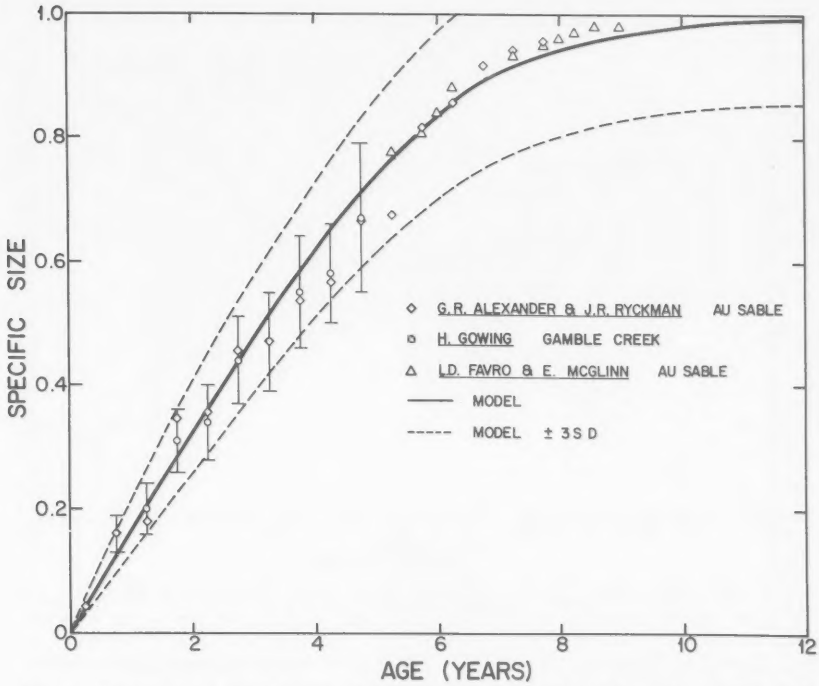


FIG. 2. Size vs. age curve corresponding to the given growth rate formula, together with the data points used to determine it. The curve and the data have been artificially normalized so that $S_\mu(\infty) = 1$. Bars on the average data points indicate the spread in the observed sizes of fish. The spread in the model is indicated by dashed lines.

the model. The low end reflects the actual current minimum growth in the Au Sable.

For mathematical simplicity we assume that the lengths of the individual fish are normally distributed about an average length $S_\mu(\sigma)$. As long as the width is chosen appropriately, a normal distribution in another variable such as weight, etc. does not have an appreciable effect on the final results. To be consistent with the experimental results of Kincaid et al. (1977) and von Limbach (1970) the spread in lengths is taken to be proportional to the square root of the length. The proportionality constant, however, is taken to be roughly 3 times larger than is necessary to fit their data. This larger constant is used to reflect the greater variability in environmental influences on the growth of wild fish as opposed to hatchery stock.

Our growth curve is displayed in Fig. 2. Since we are interested in long-term effects, seasonal variations in growth were omitted.

Mortality Rate

Despite the excellent work of Alexander (1977), data on the sources of mortality of trout in the Au Sable system are still sketchy. A large fraction, in some cases even the largest fraction, of the apparent annual loss of fish is listed as due to "unknown causes." Therefore in our model we first chose an overall mortality rate that was the same order of magnitude as the apparent rate (determined by stream-shocking surveys) and then, with subsequent calculations, we adjusted the overall rate so that the calculated population curves matched, as nearly as possible, the actual curves.

The additional mortality due to fishing pressure (when the fish exceed the legal size limit) is chosen to be 10, 20, 30 and 40% of the natural mortality. These figures are in the general range indicated by Alexander's data and were chosen to show the effects of variations in the fishing pressure. Calculations were carried out with size limits ranging from 15 to 40 cm in 5-cm steps. Historically, the size limits on various branches of the Au Sable have been in the lower half of this range and currently stand at 10 or 12 inches (taken as 25 or 30 cm, respectively) depending on location. Calculations with the higher size limits are included for the sake of completeness. Alexander's data indicate that for the critical 1–5-yr age-group the mortality rate is essentially independent of age. The excess

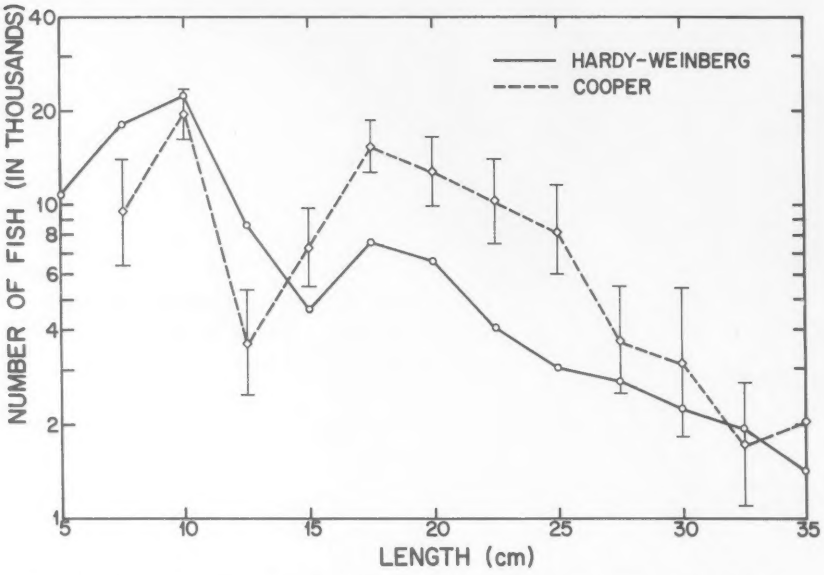


FIG. 3. Population curve corresponding to the initial Hardy–Weinberg distribution along with points derived from the empirical data of Cooper (1959). The bars on the observed numbers indicate an estimate of the probable error (i.e. expected standard deviation) in them. Both the computed curve and the observed points are normalized to give numbers of fish per 100 000 fall (i.e. September) population.

mortality rate of fry is assimilated into the fecundity rate in our model. Variations in the mortality rate of older fish, because of their relatively small population, are of little consequence in breeding. The actual yearly mortality rate, η, used in the model is given by

$$\eta = 1 - \exp(-a)$$

for fish smaller than the size limit and

$$\eta = 1 - \exp(-a - b\mu)$$

for fish above the size limit. The values of a and b are given by $a = .75$ and $b = .024$, .052, .087 and .13, respectively, for a fishing pressure mortality of 10, 20, 30 and 40% of the natural mortality. As noted above this value of a was chosen so that the overall mortality rate is in agreement with the data of Alexander. The b values are chosen such that average additional mortality for fish above the size limit is the specified percentage. The parameter μ is defined in Table 1.

No compensatory survivorship has been added for those fish that escape angling mortality in spite of some evidence (Alexander 1977) that this occurs in nature. The reason is that the extent and the degree of selectivity of this survivorship is not known. Since the fish that escape angling tend to be slow-growers, a nonselective compensatory survivorship would tend to favor the genetic stunting of the population by allowing them to contribute more to future generations.

Again no compensatory growth has been included for the surviving fish since its degree of selectivity is also unknown. However, should both of the effects be present and nonselective they would tend to neutralize each other. The survivorship would tend to decrease the average size while the change in growth rate would tend to increase it.

Results

To obtain numerical results we must first have a population with which to initiate the program. Ideally, one would like to begin with the genetic makeup of a wild population that has never been affected by human intervention. However, not only is the genetic structure of a wild population unknown, but it is also clear that such a population of *S. trutta* never existed on this continent. As a result, we have attempted instead to find a genetically stable Hardy–Weinberg distribution which is consistent with the earliest reliable data on the population of the Au Sable mainstream (see the work of Cooper 1959). We have found that the general features of the data can be described with a genetic distribution that is symmetric in each of the pairs of alleles (i.e. with both a_1 and a_2 equally likely and the same for b_1 and b_2) and with an age distribution that is determined by the known average mortality rate. The

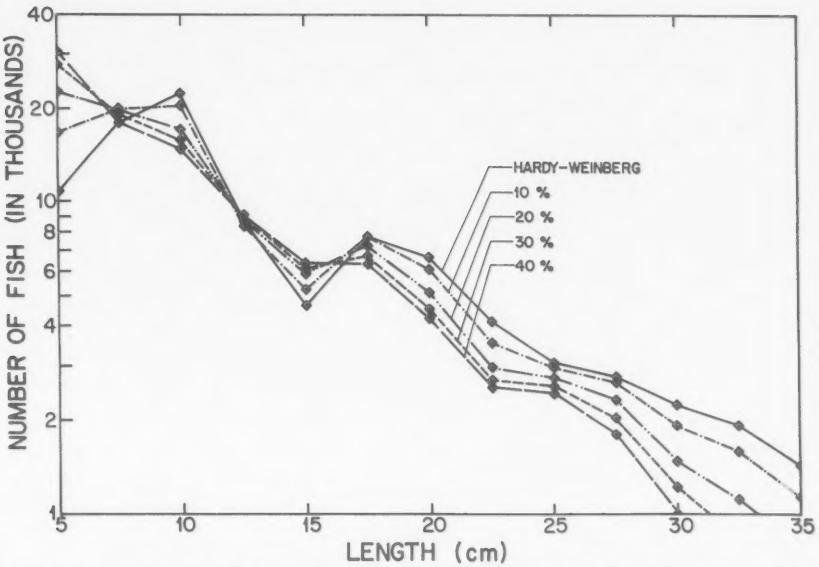


FIG. 4. Expected population numbers normalized to 100 000 after 20 yr for various amounts of fishing pressure assuming a size limit of 25 cm.

resulting population curve is plotted in Fig. 3.

Several features of this curve bear further comment. First, we observe that both the observed population and the computed curve have peaks ~10 and 18 cm. These peaks merely reflect the presence of two more-or-less distinct year-classes of fish (zero and one) at about these sizes. Second, the observed points lie above the computed curve for large (>14-cm) sizes and below the curve for small (<14-cm) sizes. This is due to a combination of our fixing the normalization (to 100 000 fish) for both curves and to the abnormally low number of 0 year-class fish present in the observed population. The decrease in the height from the 0 year to 1 year peaks in the observed population is too small to be consistent with the yearly average mortality rate alone. The low height of the 0 year peak must represent either a particularly poor spawning success the previous year or a particularly high mortality during the previous spring and summer. Since the 0 year-class represents a rather large fraction of the total population, a decrease in this class causes other portions of the population to be overemphasized when referred to a fixed total population (i.e. 100 000).

Third, the downward slope of the Hardy–Weinberg curve is definitely less than that of the observed population. That is, the observed population decreases more rapidly with increasing size than the computed population. This is to be expected since the observed population reflects the effects of previous selective fishing (which favors smaller sizes) while the Hardy–Weinberg distribution is appropriate only in situations *without* a size-dependent selection process.

Using the above Hardy–Weinberg distribution as an initial distribution we have calculated the size distribution of the expected population 5, 10, 15, 20 and 30 yr later with various size limits and amounts of fishing pressure. The size limits range from 15 to 40 cm in 5-cm increments. As noted above, fishing pressure is described in terms of a percentage increase over the natural mortality and is assigned the values 10, 20, 30 and 40%. Thus if the natural mortality rate were, say 60%/yr, 10% fishing pressure would mean a total mortality of 66%/yr, etc. Selected features of the computed results are presented in Fig. 4–7. More complete numerical results are available from the authors upon request.

Figure 4 gives the expected population numbers for various amounts of fishing pressure 20 yr after starting with the Hardy–Weinberg distribution with a 25-cm limit. As expected, increasing fishing pressure causes increased depression of the population at sizes larger than the limit. An unexpected feature is that the model indicates a depression of the population down to ~17 cm, a full 30% below the size limit. This is the result of the fact that the decline is genetic in origin and thus does not directly reflect the numerical size limit. The exact numerical value of this crossover point (~17 cm) should not be taken too literally, because it is somewhat sensitive to the details of the genetic model. However, it does indicate the general behavior to be expected

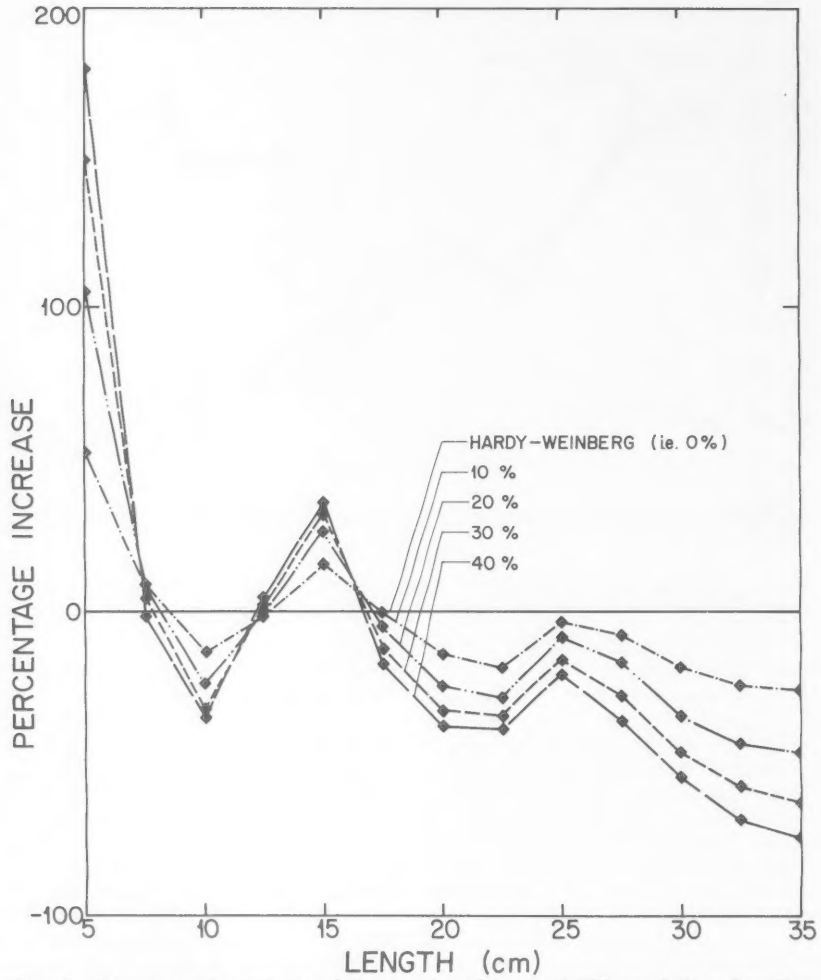


FIG. 5. Percentage change in population numbers from the initial population after 20 yr for various amounts of fishing pressure assuming a size limit of 25 cm.

from such models. Since these population numbers are given per hundred thousand, the result of this depression of the larger sizes is that the smaller size numbers are inflated in the distribution. The inflation is excessive for very small sizes (<7 cm). This excess is apparently due to the fact that our growth curve does not adequately represent the growth rate of very small fish and should be regarded as an artifact of the model. Figure 5 presents the same information as Fig. 4 but population numbers are represented as a percentage change from the initial Hardy–Weinberg numbers.

While Fig. 4 and 5 show the effect of variable fishing pressure at a fixed size limit (25 cm) and time (20 yr), Fig. 6 illustrates the effect of the variation of the size limit with a fixed fishing pressure (20%) and time (20 yr). As one would anticipate, decreasing the size limit causes a more severe decline in the numbers of larger fish. Again we see that the decline of the population begins in the neighborhood of 16–17 cm rather than near the size limit. The last calculated curve, Fig. 7, shows the effect of increasing time with a fixed limit and fishing pressure. The number of larger fish are seen to decline steadily with time and the characteristic 16–17-cm crossover is seen to show up as early as 5 yr after the onset of fishing pressure.

Figure 8 is a superposition of a calculated population ratio curve and data from the Michigan Department of Natural Resources. During most of this 20-yr period

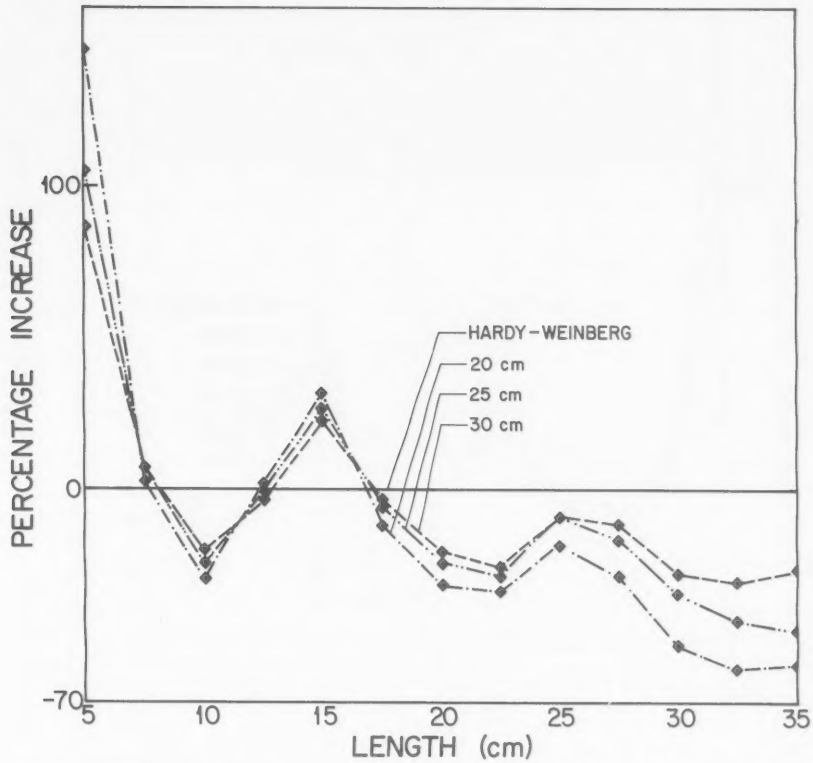


FIG. 6. Percentage change in population numbers from the initial population after 20 yr for various size limits assuming a fishing pressure of 20%.

the size limit on this stretch has been 25 cm (changed to 30 cm in 1973) and during all of this period it has been restricted to fly-fishing only so that there has been minimal kill of undersized fish returned to the water. There is a remarkable similarity between the calculated change and the actual change in the population. The observed population exhibits not only the characteristic 17-cm crossing point but also the same 15-cm peak and 10-cm minimum with a sharp peak at very small sizes (<8 cm). The exaggeration of the two observed small size peaks over the calculated curves probably stems from two sources. First, as we indicated before, Cooper's data appear to have an anomalously low number of 0 year-class fish which in turn would cause an anomalously high percentage change in this number relative to an average year. Second, and again as we have indicated before, our growth curve appears to underestimate the growth rate of very young fish so that our 0 year-class fish peak at a lower size than is observed in the natural population. It is also possible that the actual fishing pressure may have caused a higher average fishing mortality rate than assumed in our calculation. This again would cause a greater emphasis in the smaller sizes.

It may be of some interest to note the actual numerical population changes in the same stretch described above. From 1959 to 1977, the population of *S. trutta* increased from ~700 to ~800 fish, or ~13%. The *biomass* of *S. trutta*, on the other hand, decreased by ~36% over this period. The only significant competing species is the brook trout (*Salvelinus fontinalis*) which suffered a decline from ~630 to ~500 (21% in number and 65% in biomass). The total population remained essentially unchanged while total biomass suffered a 40% decrease.

Conclusions and Discussion

While the results obtained above were derived using a particular genetic model some general conclusions can be drawn from them. First, the agreement between the observed and calculated numbers for the actual population, Fig. 3, indicates that except for some minor differences at very small sizes, the expressions we have

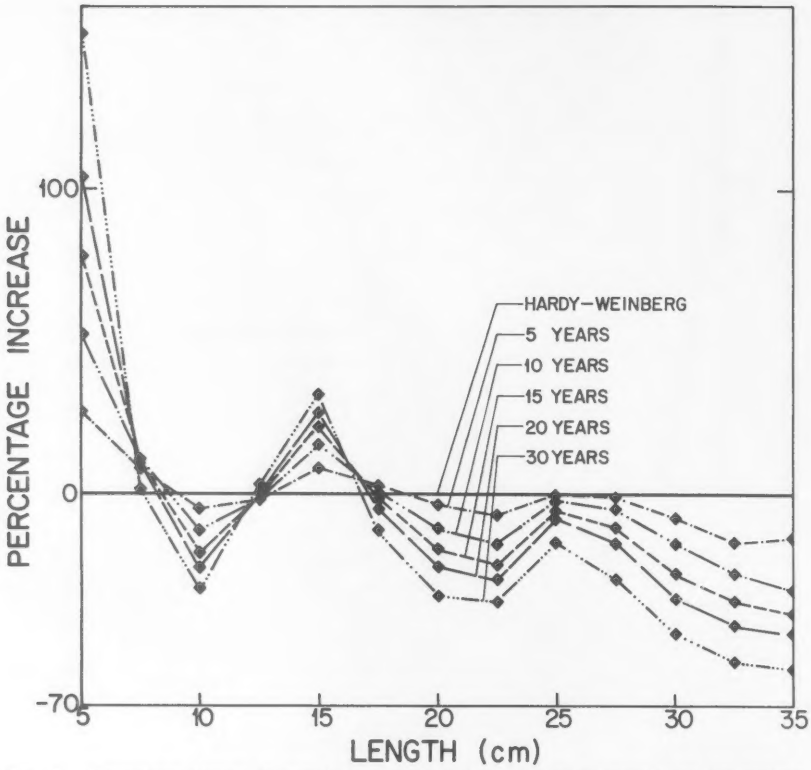


FIG. 7. Percentage change in population numbers from the initial population for various times. Size limit and fishing pressure are 25 cm and 20%, respectively, for all curves.

used for growth and mortality rates adequately describe the actual numbers. Second, the very close agreement in both shape and size between the calculated and observed population ratio curves, Fig. 8, indicates that a genetic model is capable of reproducing the observed changes in the population. It should not be concluded, however, that this agreement implies that the population changes that have taken place are necessarily genetic in origin, or if they are genetic, that our model is unique in its ability to describe them. What does seem certain is that the population of *S. trutta* in the Au Sable has a lower growth rate than was previously the case. Our model ascribes this depression of the growth rate to a selection process operating on a strictly heritable trait. An alternative genetic mechanism might be a general loss of fitness resulting from inbreeding homozygosity within a local population. (For a discussion of this effect see the recent papers by Kincaid [1976a, b] and the references therein.)

The purpose of this paper is not to present a critical evaluation of these, or other, explanations of the growth rate depression. Instead, it is to point out that the possibility of genetic effects should not be ruled out without serious consideration. With realistic input parameters (growth rate, fishing pressure, mortality, etc.), the expected magnitude of genetic effects is well within the range of the observed effects.

Finally, we should point out that sample computations have been carried out with variations on the assumptions in the genetic model. In particular, we have varied the initial genotype distribution and have obtained results very similar to the ones presented here. We have also found that the calculated results are insensitive to small variations of the growth curves. Furthermore, when one gene is neutralized to produce one-locus results, the size distribution curves are similar to the two-locus curves except they do not vary as smoothly. We have not carried out computations with multi-locus or multi-allele systems.

Acknowledgments

We thank Professor M. Petras and Dr H. L. Kincaid for their helpful comments on this manuscript, and Mr J. Samaan

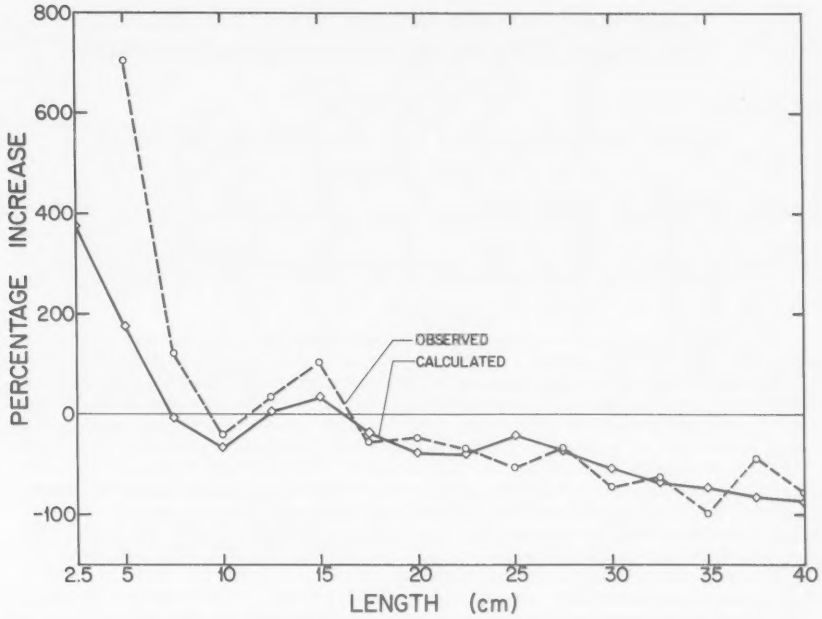


FIG. 8. Calculated population ratio curve and population ratio curve corresponding to data of Cooper (1959) and to data obtained by shocking crews of the Michigan Department of Natural Resources, for September 1977. Calculated curve is for 40% fishing pressure, 25-cm size limit, over a 20-yr period. Dashed curve represents percentage change in shocking-survey estimates of the population over an 18-yr span from fall of 1959 to fall of 1977. Note that vertical scales above and below zero are different. Both sets of data are for the stretch labeled "Wa Wa Sum."

for his invaluable assistance with the computer programming. We thank Mr W. Buc of the Michigan Department of Natural Resources, Grayling Field Office, for providing us with the raw field data for Fig. 8. This study was partially supported by a grant (No. A4625) from the National Research Council of Canada.

ALEXANDER, G. R. 1977. Consumption of small trout by large predatory brown trout in the North Branch of the Au Sable River, Michigan. Mich. Dep. Nat. Resour. Fish. Res. Rep. 1855.

ALEXANDER, G. R., AND J. R. RYCKMAN. 1976. Trout production and catch under normal and special angling regulations in the North Branch of the Au Sable River, Michigan. Mich. Dep. Nat. Resour. Fish. Res. Rep. 1840.

AULSTAD, D., T. GJEDREM, AND H. SKJERVOLD. 1972. Genetic and environmental sources of variation in length and weight of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 29: 237–241.

BRIDGES, W. R. 1973. Fish genetics laboratory, p. 61–66. *In* Progress in sport fishery research 1971. U.S. Fish Wildl. Serv. Resour. Publ. 121.

COOPER, G. P. 1959. Mich. Dep. Nat. Resour. Fish. Res. Rep. 1577.

DONALDSON, L. R., AND D. MENASVETA. 1961. Selective breeding of chinook salmon. Trans. Am. Fish. Soc. 90: 160–164.

GOWING, H. 1975. Population dynamics of wild brown trout in Gamble Creek, subject first to angling then with no angling. Mich. Dep. Nat. Resour. Fish. Res. Rep. 1824.

KINCAID, H. L. 1972. A preliminary report of the genetic aspects of 150-day family weights in hatchery rainbow trout. Proc. 52nd Annual Conf. West. Assoc. State Game Comm., Portland, Oreg.

1976a. Inbreeding in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 33: 2420–2426.

1976b. Effects of inbreeding on rainbow trout populations. Trans. Am. Fish. Soc. 105: 273–280.

KINCAID, H. L., W. R. BRIDGES, AND B. VON LIMBACH. 1977. Three generations of selection for growth rate in fall-spawning rainbow trout. Trans. Am. Fish. Soc. 106: 621–628.

VON LIMBACH, B. 1970. Fish genetics laboratory, p. 110–117. *In* Progress in sport fishery research. 1969. U.S. Fish Wildl. Serv. Resour. Publ. 88.

Infectious Pancreatic Necrosis Virus: Quantification of Carriers in Lake Populations During a 6-Year Period

T. YAMAMOTO

Department of Microbiology, University of Alberta, Edmonton, Alta. T6G 2E9

AND J. KILISTOFF

Department of Indian and Northern Affairs, Parks Canada, Western Region, Calgary, Alta. T6G 0X5

YAMAMOTO, T., AND J. KILISTOFF. 1979. Infectious pancreatic necrosis virus: quantification of carriers in lake populations during a 6-year period. J. Fish. Res. Board Can. 36: 562–567.

An infectious pancreatic necrosis (IPN) carrier population of brook trout (*Salvelinus fontinalis*) planted in an isolated natural lake was tested for virus over a period of 6 yr. The initial isolation rate of 90% had decreased to 50% during this period indicating that there is a prolonged persistence of virus in infected animals of this species. The ceca with the associated pancreatic tissue of these fish yielded virus most frequently during the early years but the virus then decreased to low levels. Kidneys were the most consistent organs to yield virus throughout the study period. The feces yielded little virus at first but later increased for several years and then decreased. The spleen and liver yielded virus infrequently. Surgically removed gonads were the least frequent yielders of virus. The low virus isolation rate from progeny of the carrier population and from other planted fish indicated that transmission in the wild is probably infrequent. Other lakes with normal angling that had plantings with IPN carrier fish but subsequently planted annually with disease-free fish had populations whose carrier rate had decreased to undetectable levels within a 6-yr period. These findings indicated that the practice of planting IPN-free fish into areas where carriers were previously introduced may lead eventually to a carrier-free state.

Key words: infectious pancreatic necrosis virus, *Salvelinus fontinalis*, virus persistence, carrier fish, virus isolation rate

YAMAMOTO, T., AND J. KILISTOFF. 1979. Infectious pancreatic necrosis virus: quantification of carriers in lake populations during a 6-year period. J. Fish. Res. Board Can. 36: 562–567.

Une population d'ombles de fontaine (*Salvelinus fontinalis*) porteuse de la nécrose pancréatique infectieuse (IPN) et introduite dans un lac naturel isolé fut suivie sur une période de 6 ans en vue de mesurer l'incidence du virus. Le taux initial d'isolation de 90% passa à 50% au cours de cette période, ce qui indique qu'il y a persistance prolongée du virus chez les sujets infectés. Les premières années, les caeca et le tissu pancréatique qui leur est associé chez ces poissons furent les endroits d'où l'on put isoler le virus le plus fréquemment, mais par la suite, le virus diminua à de bas niveaux. Les reins furent les organes à fournir le plus uniment des virus durant toute la période d'étude. Les fèces donnèrent peu de virus au début mais augmentèrent durant plusieurs années subséquentes, pour ensuite diminuer. La rate et le foie ne donnèrent le virus que rarement. Les gonades enlevées par chirurgie étaient les organes où le virus se trouvait le moins fréquemment. Le faible taux d'isolation du virus chez les descendants de la population porteuse et chez d'autres poissons ensemencés indique que la transmission dans la nature est probablement rare. D'autres lacs où se pratiquait une pêche sportive normale et dans lesquels on avait introduit des peuplements de poissons porteurs de l'IPN mais qui, par la suite, avaient reçu annuellement des poissons sains contenaient des populations chez lesquelles la proportion de porteurs avait diminué et atteint des niveaux indétectables durant une période de 6 ans. Ces résultats indiquent que le peuplement de poissons exempts d'IPN dans des régions où l'on avait introduit des poissons porteurs peut éventuellement conduire à une situation où il n'y a plus de porteurs.

Received August 14, 1978
Accepted January 25, 1979

Reçu le 14 août 1978
Accepté le 25 janvier 1979

THE problem of infectious pancreatic necrosis (IPN) in carrier fish in lakes and streams is of considerable concern with respect to the possible transmission of infection into hatcheries that depend on surface water supplies. In certain areas such as the Maritime provinces it has been shown that many of the fish culture stations have IPN carrier populations (MacKelvie and Artsob 1969). Because all carrier fish are asymptomatic, there are no means of identifying them short of

Printed in Canada (J5345)
Imprimé au Canada (J5345)

TABLE 1. Lakes in Banff and Jasper National Parks netted for IPN carrier fish.

Lake	Lat.	Long.	Altitude (m)	Max. depth (m)	Avg. depth (m)	Surface area (km^2)	Species of fish present[a]
Annette	52°54′ N	118°02′ W	1024	23	6.6	0.29	BT, RT
Beauvert	52°52′ N	118°03′ W	1030	25	6.8	0.32	BT, RT, DV, WS, Ling, NP, MW
Beaver	52°51′ N	117°43′ W	1498	2.0	1.2	0.31	BT, RT
Buck	52°33′ N	117°40′ W	1372	7.3	1.5	0.12	BT, RT
Edith	52°54′ N	118°02′ W	1024	18	5.1	0.50	RT
Patricia	52°54′ N	118°05′ W	1180	40	12	0.69	BT, RT, LT, Ling
Pyramid	52°55′ N	118°05′ W	1186	19	8.7	1.27	BT, RT, LT, ST, MW, WS
Johnson	51°11′ N	115°28′ W	1402	5.2	2.4	0.12	BT, RT, WS
Upper Vermilion	51°10′ N	115°37′ W	1387	4.0	1.5	0.11	BT, RT, CT, DV, MW, WS

[a]BT = brook trout (*Salvelinus fontinalis*); RT = rainbow trout (*Salmo gairdneri*); DV = dolly varden (*Salvelinus malma*); WS = white sucker (*Catostomus commersoni*); Ling = burbot (*Lota lota*); NP = northern pike (*Esox lucius*); MW = mountain whitefish (*Prosopium williamsoni*); LT = lake trout (*Salvelinus namaycush*); ST = splake trout (brook trout × lake trout); CT = cutthroat trout (*Salmo clarki*).

direct isolation of the virus (Billi and Wolf 1969; Yamamoto 1975a) or possibly by some serological means such as the fluorescent antibody technique (Piper et al. 1973, Tu et al. 1974).

Studies with respect to the incidence of carrier fish in populations vary depending on the species of fish involved. Billi and Wolf (1969) found that almost all brook trout (*Salvelinus fontinalis*) surviving on IPN epizootic were carriers upon repeated examination of feces and peritoneal washes over a period of 9 mo. In comparison rainbow trout (*Salmo gairdneri*) appeared to have a lower carrier rate (Wolf et al. 1968). Similarly preliminary studies indicated that brook trout have a high carrier incidence (Yamamoto 1975a) but rainbow trout have a lower and rapidly decreasing carrier rate (Yamamoto 1975b).

Our study of IPN in brook trout and to a lesser extent in other salmonids, was conducted over a period of 6 yr to determine the virus isolation rate from a population in an enclosed protected lake. The study is compared to data obtained from lakes that had been previously planted with IPN carriers followed by annual plantings of fingerling-sized fish and subject to normal angling pressures.

Materials and Methods

Lakes chosen for this study had been planted with IPN carriers during 1972 and probably earlier. Buffalo Lake measuring ~0.04 km^2 in surface area and located (49°07′N, 113°51′W) in Waterton Lakes National Park in the southwest corner of the province of Alberta was an isolated natural lake with no surface inlet or outlet. In certain years when the water level is high water flows from an upper lake into Buffalo Lake. In the spring of 1972, 2000 2½-yr-old brook and 200 rainbow trout from the Maligne River Hatchery at Jasper National Park were planted in Buffalo Lake. Shortly thereafter it was established that the lot of brook trout from which the plantings had been made were IPN carriers to at least 90% of the population (Yamamoto 1974). Because of the high IPN carrier status of the brook trout, continued monitoring for the virus in the fish population in Buffalo Lake was proposed. Although the lake had been planted in previous years, winter kills left few if any survivors prior to planting in 1972. The massive imposition of a known carrier population on an assumed small number of resident trout provided an opportunity to evaluate annually the virus isolation rate of the planted fish and the extent to which new carriers were produced.

In addition to Buffalo Lake, nine lakes located in Jasper and Banff National Parks in Alberta were selected for IPN testing because they also had been planted with IPN carriers from the Maligne River Hatchery. The management of the fish population in these lakes was different to that of Buffalo Lake in that angling was permitted and virus-free fingerlings planted annually. Data on these lakes are given in Table 1.

Fish for IPN virus isolation were obtained from Buffalo Lake by angling and by gill netting. Fish from other lakes were also obtained by gill netting with different sizes of mesh. Fish were aged by standard scale or otolith determinations.

For virus isolation ~0.2 g of tissue was removed aseptically from kidneys, liver, spleen, and gonads and placed on ice. The organs were ground and suspended in 0.85% saline containing 1000 IU penicillin, 1000 μg streptomycin, and 1000 μg kanamycin sulfate per millilitre. One tenth millilitre of the supernatant was inoculated into 16-mm tube cultures of chinook salmon embryo cells (CHSE-214) or alternatively into 15-mm diam wells (Tissue Culture Cluster, Costar, Cambridge, Mass). Cultures were incubated at 18°C in an atmosphere of 5% CO_2 in air. Cultures were grown in Minimum Essential Medium (Eagle) containing 1% fetal bovine serum, 100 IU penicillin, and 100 μg streptomycin/millilitre. Cultures were examined at regular intervals for 14 d. Medium from cultures showing changes was diluted and transferred twice to determine whether cytopathic effect was due to virus or to toxicity.

Tests for virus identification included resistance to ether, neutralization with specific IPN antiserum, and electron microscopy using the methods of Yamamoto (1974) and Chang et al. (1978).

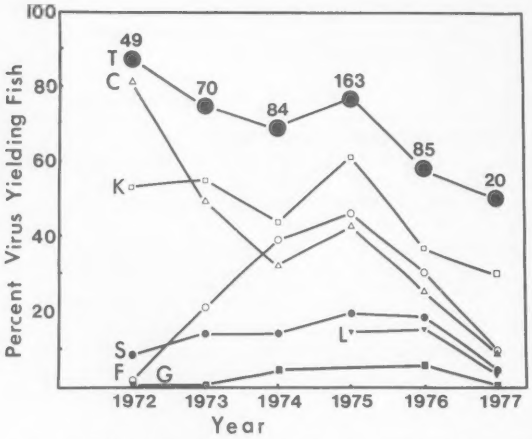


FIG. 1. IPN virus isolation rates from the brook trout carrier population in Buffalo Lake. Number above the points indicates number of fish examined. T = total IPN virus-yielding fish. C, K, S, F, G, and L refer to ceca, kidneys, spleen, feces, gonads, and liver, respectively.

Results

IPN Virus Isolation from the Carrier Population Planted in Buffalo Lake

During 1972, forty-nine 2½-yr-old brook trout from the Maligne River Hatchery, were examined for IPN virus at the time of stocking Buffalo Lake. Therefore these fish were considered to be representative of the population that were placed in Buffalo Lake. The ceca, kidneys, spleen, feces, and gonads were examined separately for virus. Some of this data was presented earlier (Yamamoto 1975a) but the complete data are presented in Fig. 1. Of the 49 fish examined 43 or 87.8% yielded virus in 1972. The ceca yielded virus most frequently with an incidence of 85%. Kidneys, spleen, feces and gonads yielded virus at rates of 50, 9, 2, and 0%, respectively.

During 1973, 53 of 70 fish planted in Buffalo Lake yielded virus to give an isolation rate of 76%. The rate from the ceca had dropped to 50% while that from the kidneys remained at about 55%. The incidence of virus occurring in the spleen increased slightly to 12% and in the feces to 22%.

During 1974 the total isolation rate had dropped slightly to 70% of the 84 fish examined but the general decline in the isolation of the virus from ceca and feces continued. In 1975, 226 fish were examined and 158 or 70% of the fish yielded virus. Of the 226 fish, 163 were chosen at random and their organs tested separately; the kidneys were found to yield virus most frequently with 60%, feces with 43%, ceca with 40%, and spleen with 20%.

During 1976 most organs showed a decline in virus content and this decrease continued until 1977 when the total isolation rate was 50%. Throughout the 1972–77 period kidneys were the most consistent yielder of virus; the ceca, although a high yielder initially, decreased to ~10% over the 5-yr period. Interestingly, the initial virus isolation rate from feces was very low but peaked 3 yr later and then declined. The spleen and the liver yielded virus infrequently and the gonads were the least productive.

We found no sex related or seasonal differences in the IPN carrier rate. Nor was there any significant difference in size whether or not the fish were virus yielders.

IPN Virus Isolations from Progeny of the Carrier Brook Trout

A large hatch of young fry resulted from the 1975 fall spawn of the carrier population. During the following spring a number of young were observed in Buffalo Lakes. Only one fingerling was caught in May 1976, but many were caught in October 1977 and during the following year. The weight gain of these progeny fish were tabulated to determine whether infection with IPN might affect the growth rate. Coincident with the successful hatch was an increase in water level of the lake that resulted in an overflow from the unstocked upper lake into Buffalo Lake. This flow of water allowed a large number of the fingerlings to migrate to the upper lake during 1976. Early in 1977 the lake level decreased and continued to do so until late fall. Therefore the data includes progeny fish netted in 1977 from both the upper and lower lakes.

A total of 2294 progeny fish, 679 from the lower and 1615 from the upper lakes, were examined for virus. Virus was isolated from 4 fish of 132 sampled during 1976 giving a rate of 3%. The four infected fish were all within the average size limits at the time they were caught. Thus infection of these fish with IPN did not appear to have any observable effect on their growth rate. The following year no isolations were made from a total of 2161 progeny fish sampled. The overall virus isolation from these progeny fish over the 2-yr period was 0.2%. This low carrier incidence contrasts with the high incidence often found in hatcheries.

Frequency of Transmission to Other Fish Planted in Buffalo Lake

In addition to the testing of the carrier population and their progeny a number of salmonids (that were known from past studies to be capable of becoming infected and carrying the virus) were planted in Buffalo Lake at varying times. These included 233 yearling rainbow trout during 1974, 230 lake trout (*Salvelinus namaycush*) and 165 mountain whitefish (*Prosopium williamsoni*) during 1975, and 200 3-yr-old brook trout in 1976. Of the 823 fish planted the total recovery of these plantings were 1.3, 10.4 and 40% for rainbow trout, lake trout, mountain whitefish, and brook trout,

TABLE 2. IPN virus isolations from brook and rainbow trout taken from lakes in Jasper and Banff National Parks as a ratio of isolations to number examined.

Species	Lake	1973	1974	1977
Rainbow trout	Annette	0/5		0/16
	Beauvert	2/7		0/0
	Beaver	0/25	0/6	0/1
	Buck	0/22		0/2
	Edith	2/18		0/13
	Patricia	6/74	4/174	0/93
	Pyramid	0/2	0/3	0/32
	Johnson	0/3		0/2
	Vermilion	0/5	0/3	0/3
Total		10/161 (6.2%)	4/186 (2.2%)	0/162
Brook trout	Annette	4/22		0/105
	Beauvert	0/0		0/0
	Beaver	10/103		1/158
	Buck	11/47	0/164	0/23
	Edith			
	Patricia	16/39	5/17	0/8
	Pyramid	4/4	0/0	0/6
	Johnson	0/48		0/30
	Vermilion	1/12	0/25	0/10
Total		46/275 (16.7%)	5/206 (2.4%)	1/340 (0.3%)
Total for both species		56/436 (12.8%)	9/392 (2.3%)	1/502 (0.2%)

respectively. Of the 79 fish examined for virus, none yielded virus. This limited experiment indicated that establishment of the carrier state among planted susceptible species occurred infrequently in Buffalo Lake.

IPN Virus Carriers in Lakes in Jasper and Banff National Parks

The selected lakes in Banff and Jasper National Parks examined for IPN virus carriers are given in Table 1 together with the species of fish present in each lake and the physical parameters of the lakes. All fish planted in these lakes after 1972 were obtained from disease-free stocks examined for virus before shipment and at the time of planting and all lots were considered to be free of IPN virus. Thus virus-yielding fish in these lakes could be those carrier stock planted during or prior to 1972 or those fish either progeny or planted that had become infected in the lake and survived as carriers. Gill nettings were conducted during the summers of 1973, 1974, and 1977. The numbers of rainbow and brook trout obtained from each of the lakes and examined for virus are presented in Table 2.

Virus detection for all the rainbow trout examined decreased from 10 out of 161 in 1973 to 4 out of 186 in 1974 to none out of 162 in 1977. The comparable values for brook trout were 46 out of 275 in 1973, 5 out of 206 in 1974, and 1 out of 340 in 1977. Using these data the chi-square value for independence calculated for rainbow trout was 11.7 and 71.5 for brook trout. Since both of these values are significant at $P > 0.01$, the hypothesis that virus detection was independent of years was rejected. Consequently it may be concluded that there was a statistically significant reduction in the carrier rate of these two species over the test period. Except for 1 fish the ages of the virus-yielding brook and rainbow trout were such that they could have been survivors of an IPN epizootic at the Maligne River Hatchery in 1972 or possibly earlier. Some of these fish had fin clip markings that indicated their hatchery origin. Since not all planted fish were marked the unmarked fish could not be identified whether they were from natural reproduction or hatchery reared.

Other fish examined for virus during this period included 80 lake trout, 30 splake trout (brook trout × lake trout), 6 dolly varden (*Salvelinus malma*), 1 cutthroat trout (*Salmo clarki*), 244 mountain whitefish, 1 northern pike (*Esox lucius*), 6 burbot (*Lota lota*), and 258 whitesucker (*Catostomus commersoni*). Of these fish 2 of 16 lake trout and 2 of 30 splake trout taken from Pyramid Lake and 1 of 65 mountain whitefish from upper Vermilion Lake were found to carry the virus. The fish of other species examined did not yield virus.

The results of virus isolations from fish in these selected lakes indicate a decreasing carrier rate in trout populations to undetectable levels within 5–6 yr when disease-free stocks are planted annually. The finding that lake trout and splake trout can be carriers appears to be a new observation. The planting records of 1972 (known epizootic year at the Maligne River Hatchery) and the ages of the virus yielding lake trout and splake trout taken from Pyramid Lake during 1974 is such that

they could have been reared at the Maligne River Hatchery and planted as infected animals. In contrast the one mountain whitefish taken from Vermilion Lake in Banff National Park must have been infected in the wild since that species has not been reared at the Maligne River Hatchery. The one fish that yielded virus in 1977 was a 2-yr-old brook trout taken from Beaver Lake. Since no infected fish were planted since 1972, this single virus isolation provided the only evidence that some transmission is occurring in the wild. The low rate of fish carrying virus would indicate that infection and persistence of IPN in the wild in either naturally reproduced or planted fish occurs infrequently.

Discussion

The data from infected brook trout planted in Buffalo Lake indicated that this species of salmonid remain infected for a long period of time. These findings confirm the persistence of this virus in an earlier study by Billi and Wolf (1969). In their study they followed the carrier state of brook trout for 9 mo and found that almost all of the fish remained persistent carriers.

In the testing of fish for IPN carriers Billi and Wolf (1969) suggested the use of a nonlethal sampling procedure, involving the examination of feces. For brood stock it appeared that the testing of reproductive materials would be useful for the detection of virus. Wolf et al. (1963) stripped brook trout of eggs and found high levels of virus in the egg fluids. In contrast the Buffalo Lake data of surgically sampled gonads of infected brook trout yielded virus infrequently. To what extent spawn material may be contaminated with feces or urine during sampling should be investigated further.

Our studies indicated that virus content of feces of infected fish varies considerably from a low of 2% to a high of 43% and is therefore not a reliable source to indicate the presence of infection.

Our studies show that the kidneys are the single most consistent yielder of virus irrespective of the age of the fish. Similar conclusions were reached by Wolf and Quimby (1971) for adult IPN carrier trout. However, the age of the trout appears to have an effect on the rates of isolation from other organs. The ceca-associated pancreatic tissue yielded virus most frequently but later decreased to minimal levels. In contrast, virus in the feces was minimal in the early years but increased for several years before decreasing again.

The finding that the ceca (with the pancreatic tissue) frequently yielded virus in the early years of the fish indicates that the pancreatic tissue is the primary organ affected by the virus (Wood et al. 1954). This finding was further extended by McKnight and Roberts (1976) who showed that the pathological effects of the virus are detectable in the pancreatic and intestinal tissue in clinically normal survivors. Apparently the carrier state can represent differing degrees and stages of infection. The changing frequency of virus isolation would also indicate changes in the histopathological state of the infection. The low isolation rate of IPN virus from Buffalo Lake brook trout progeny indicated that transmission and infection in the wild does not readily occur. It could be argued that a large proportion of the infected animals did not survive as carriers but at no time were there any dead or ill progeny brook trout observed. In contrast, under crowded conditions in hatcheries, epizootics can occur with infection rates approaching 100% (Snieszko et al. 1959; Billi and Wolf 1969; Frantsi and Savan 1971; Yamamoto 1974). This difference in IPN incidence indicates that virus is not readily transmitted vertically from carrier brood stock with the egg. However, evidence of the failure to disinfect eggs taken from carrier brood stock can be interpreted to indicate that some eggs could be infected (Wolf et al. 1963; Bullock et al. 1976).

The marked reduction in IPN isolations following the distribution of carriers into lakes in Jasper and Banff National Parks indicates that the infection of young fish does not readily occur. This finding is further supported by a recent newsletter report in the Fish Health Section of the American Fisheries Society (Vol. 6, No. 1, p. 5–6, 1978) where the practice of distributing IPN-free fish into a lake below the Colorado River since 1970 had effectively diluted the carriers to undetectable levels. The question is whether the incidence of disease will continue to decrease and whether it can become nonexistent in water sheds where it has been introduced. Obviously this is a question that has no definite answer because of the limits of detectability. The other question is whether surface waters can be used for hatchery operation without an IPN epizootic. There is always the probability that a single virus, if it infects a fry, can be a nucleus for an epizootic and eventually destroy an entire year's production. If surface water for hatchery use is a necessity in areas previously contaminated, it would be advisable not to do so for a period of 5 yr to reduce the number of carriers and then to disinfect waters for egg incubation and the rearing of fry. Generally speaking the older fish, being less crowded, would be less prone to being infected and to spread IPN virus to younger fish.

Some years ago Wolf (1972) in a review on fish virology stated that "Hatcheries which use stream or other water sources harboring fish have virtually insurmountable obstacles and, except by sterilizing the water, it would not be possible to avoid exposure to infection." It would seem that the IPN problem may not be insurmountable in polluted areas if certain practices are exercised. The current findings would indicate that a program of IPN eradication by avoiding for 5–6 yr contaminated water supplies to prevent the infection of young fry and fingerlings combined with the policy of planting healthy noncarrier stock could eventually lead to virus-free water supplies for hatchery operations. This practice, which promotes the health of fish by avoidance, would appear to be more desirable than the

wide distribution of so-called "attenuated" vaccines whose behavior in carrier populations could lead to unpredictable problems.

Acknowledgments

We thank Parks Canada, Western Region, Calgary, for promoting this study and providing funds throughout the years for this prolonged study. Much of the study was also supported by grants from the Department of the Environment, Fisheries and Marine Service. Grateful acknowledgment is expressed to the Wardens' Service of the Western Parks Region, to the many technicians and summer assistants involved in the study, and to L. W. Thomson and P. Fedorak for statistical analysis.

BILLI, J. L., AND K. WOLF. 1969. Quantitative comparison of peritoneal wastes and feces for detecting infectious pancreatic necrosis (IPN) virus in carrier brook trout. J. Fish. Res. Board Can. 26: 1459–1465.

BULLOCK, G. L., R. R. RUCKER, D. AMEND, K. WOLF, AND H. M. STUCKEY. 1976. Infectious pancreatic necrosis: transmission with iodine-treated and nontreated eggs of brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 33: 1197–1198.

CHANG, N., R. D. MACDONALD, AND T. YAMAMOTO. 1978. Purification of infectious pancreatic necrosis (IPN) virus and comparison of polypeptide composition of different isolates. Can. J. Microbiol. 24: 19–27.

FRANTSI, C., AND M. SAVAN. 1971. Infectious pancreatic necrosis virus: comparative frequencies of isolation from feces and organs of brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 28: 1064–1066.

MACKELVIE, R. M., AND H. ARTSOB. 1969. Infectious pancreatic necrosis virus in young salmonids of the Canadian Maritime Provinces. J. Fish. Res. Board Can. 26: 3259–3262.

MCKNIGHT, I. J., AND R. J. ROBERTS. 1976. The pathology of infectious pancreatic necrosis. 1. The sequential histopathology of the naturally occurring condition. Br. Vet. J. 132: 76–85.

PIPER, D., B. L. NICHOLSON, AND J. DUNN. 1973. Immunofluorescent study of the replication of infectious pancreatic necrosis virus in trout and Atlantic salmon cell cultures. Infect. Immun. 8: 249–254.

SNIESZKO, G. F., K. WOLF, J. E. CAMPER, AND L. L. PETTIJOHN. 1959. Infectious nature of pancreatic necrosis. Trans. Am. Fish. Soc. 88: 289–293.

TU, K.-C., R. S. SPENDLOVE, AND R W. GOEDE. 1974. Immunofluorescent cell assay of infectious pancreatic necrosis virus. Appl. Microbiol. 27: 593–599.

WOLF, K. 1972. Advances in fish virology: A Review 1966–1971, p. 305–331. *In* Diseases of fish. Symp. Zool. Soc London 30.

WOLF, K., AND M. C. QUIMBY. 1971. Salmonid viruses: Infectious pancreatic necrosis virus. Morphology, pathology and serology of first European isolations. Arch. Gesamte Virusforsch. 34: 144–156.

WOLF, K., M. C. QUIMBY, AND A. D. BRADFORD. 1963. Egg-associated transmission of IPN virus of trouts. Virology 21: 317–321.

WOLF, K., M. C. QUIMBY, C. P. CARLSON, AND G. L. BULLOCK. 1968. Infectious pancreatic necrosis: detection of virus free stock from a population of carrier trout. J. Fish. Res. Board Can. 25: 383–391.

WOOD, E. M., S. F. SNIESZKO, AND W. T. YASUTAKE. 1954. Infectious pancreatic necrosis in brook trout. Arch. Pathol. 60: 26–28.

YAMAMOTO, T. 1974. Infectious pancreatic necrosis virus occurrence at a hatchery in Alberta. J. Fish. Res. Board Can. 31: 397–402.

1975*a*. Frequency of detection and survival of infectious pancreatic necrosis virus in a carrier population of brook trout. (*Salvelinus fontinalis*) in a lake. J. Fish. Res. Board Can. 32: 568–570.

1975*b*. Infectious pancreatic necrosis (IPN) virus carriers and antibody production in a population of rainbow trout (*Salmo gairdneri*). Can. J. Microbiol. 21: 1343–1347.

Short-Term Covariability of Chlorophyll and Temperature in the St. Lawrence Estuary[1]

S. DEMERS, P. E. LAFLEUR, L. LEGENDRE, AND C. L. TRUMP[2]

Département de biologie, Université Laval, Québec, Qué. G1K 7P4

DEMERS, S., P. E. LAFLEUR, L. LEGENDRE, AND C. L. TRUMP. 1979. Short-term covariability of chlorophyll and temperature in the St. Lawrence Estuary. J. Fish. Res. Board Can. 36: 568–573.

Time series of chlorophyll *a* fluorescence and temperature taken at a depth of 1 m were recorded at 4-min intervals for 136.5 h, at an anchor station located in the Middle Estuary of the St. Lawrence. The sampling program was carried out during the transition from neap tides to spring tides. Power spectral analysis was applied to the series and variance spectra were estimated. Coherency spectra between fluorescence and temperature were also computed. Results indicate that chlorophyll distribution was controlled by physical processes during neap tides. Conversely, lesser amounts of chlorophyll *a* per cell and lesser primary production rates were noted during spring tides. A flattening of the chlorophyll spectrum was observed for this part of the study. This result was interpreted in terms of the poor physiological state of the phytoplankton population that was sampled during the second part of the study, a different density structure being present due to the change in tidal energy.

Key words: time series, chlorophyll, temperature, spectral analysis, St. Lawrence Estuary

DEMERS, S., P. E. LAFLEUR, L. LEGENDRE, AND C. L. TRUMP. 1979. Short-term covariability of chlorophyll and temperature in the St. Lawrence Estuary. J. Fish. Res. Board Can. 36: 568–573.

Des séries de données de fluorescence de la chlorophylle *a* et de température prises à 1 m sous la surface ont été enregistrées à intervalles de 4 min pendant 136.5 h, à une station fixe située dans l'estuaire moyen du St-Laurent. L'enregistrement fut réalisé durant la transition des marées de morte-eau aux marées de vive-eau. Ces séries furent soumises à l'analyse spectrale en vue d'estimer les spectres de variance. D'autre part, on a évalué des spectres de cohérence entre les enregistrements de fluorescence et de température. La similitude entre les spectres de chlorophylle et de température indique que les facteurs physiques du milieu exercent un contrôle sur la distribution de la chlorophylle au cours des marées de morte-eau. En marées de vive-eau, on observe un aplatissement du spectre de variance de la chlorophylle, alors que l'on remarque des concentrations plus faibles de chlorophylle *a* par cellule que lors des marées de morte-eau, ainsi que des taux inférieurs de production primaire. Cette situation est interprétée en relation avec l'état physiologique manifestement déficient de la population phytoplanctonique échantillonnée pendant la deuxième partie de l'étude, la structure de la colonne d'eau étant alors différente par suite du changement de l'énergie des marées.

Received July 28, 1978
Accepted January 22, 1979

Reçu le 28 juillet 1978
Accepté le 22 janvier 1979

SINCE the exploratory work of Bainbridge (1957), it is generally recognized that planktonic organisms display patchiness in their distribution. Such "patches," as demonstrated by Platt et al. (1970) in a nearshore environment off Nova Scotia through autocorrelation analysis, can have characteristic diameters of ~1 naut. mile (1850 m). In the St. Lawrence Estuary, Fortier et al. (1978), using the same mathematical approach, reported patches of ~750 m diam. Applying power spectral analysis, Platt (1972) found a variance spectrum of the −5/3 power-law type, for scales between 10 and 1000 m. This observation suggested a possible control of chlorophyll distribution by a turbulence related mechanism, a −5/3 slope in the turbulence spectrum being predicted for isotropic turbulence (Kolmogorov 1941). There may be large variations in the range over which chlorophyll remains under physical control. In Lake Tahoe, Powell et al. (1975) noted a marked similarity between chlorophyll and current spectra, but unlike Platt's (1972) observation, this similarity held over smaller length scales (<100 m). Fasham and Pugh (1976), after sampling chlorophyll and temperature in the open ocean, suggested that the distance over which the distribution of phytoplankton is related to physical processes may vary in length in different areas of world oceans. The coherence between chlorophyll and tem-

[1]Contribution to GIROQ program (Groupe interuniversitaire de recherches océanographiques du Québec).

[2]Authors listed alphabetically.

Printed in Canada (J5325)
Imprimé au Canada (J5325)

perature seems to be high for shorter length scales in the tropics than in temperate regions. Recently, Lekan and Wilson (1978), working in Long Island Sound, observed that the spectra of chlorophyll and physical parameters (temperature and salinity) were quite similar for length scales from 5 to 20 km, whereas at larger scales, chlorophyll distribution was related to the amount of nutrients (mainly ammonium). At scales <5 km, a −1 slope was observed in the chlorophyll spectrum, and this was related to the growth of phytoplankton.

Most workers have investigated the variability of chlorophyll along the horizontal axis whereas the vertical component was generally neglected. Denman and Platt (1975) and Denman (1976) observed a positive correlation between chlorophyll and temperature series, but no correlation between two chlorophyll records collected at depths 4 m apart. Their results suggested that part of the variability observed in constant-depth records could be related to the action of internal waves moving along vertical gradients.

In 1975, GIROQ began to investigate the variability in the St. Lawrence Estuary through the use of time series analysis (ECOVARIATE program). The present paper focuses on an in vivo chlorophyll *a* fluorescence and a simultaneous temperature series, in an attempt to elucidate the relationships between these two parameters in an estuarine environment.

Materials and Methods

This study was made in the St. Lawrence Estuary, in an area denoted as the Middle Estuary (Fig. 1). This area extends from Ile d'Orléans, near Quebec City, to the mouth of the Saguenay River, ~150 km downstream. The Middle Estuary has a shallow plateau on the southern side and a basin-and-sill configuration on the northern side from the Saguenay to Pointe-au-Pic. Upstream, the estuary is relatively shallow. The sampling was carried out at an anchor station in the center of the Middle Estuary, near Point-au-Pic (70°04′W, 47°39′N) from July 5 to 12, 1975. This station is located at the upstream end of a basin, where the water is 50 m deep. This region of the Middle Estuary can be classified as "partially mixed," the average salinity gradient being about 10‰ from the surface to the bottom.

Chlorophyll *a* fluorescence was measured continuously using a Turner model 111 fluorometer, as modified in the method of Lorenzen (1966). A pump provided the fluorometer with water from 1 m below the surface. The record was discretely sampled every 4 min and data stored on magnetic tape. To verify the relationship between fluorescence and the concentration of chlorophyll *a*, a 1-L water sample was taken every hour from the outflow of the fluorometer, and chlorophyll *a* concentration was determined according to the fluorometric method (Strickland and Parsons 1972). The fluorescence–chlorophyll *a* relationships were estimated separately for day and night by model II regression (Sokal and Rohlf 1969), which is appropriate when both variables are subject to experimental error, and were not significantly different ($P > 0.05$). Therefore, an overall coefficient of linear correlation was computed and found significant ($r = 0.52$, $P < 0.001$). Simultaneously with fluorescence, temperature was recorded at depths of 1 and 10 m on magnetic tape. An Aanderaa current meter, equipped with a thermistor and suspended at a depth of 2 m off the stern of the ship, also recorded temperature data during the first 76 h of the program.

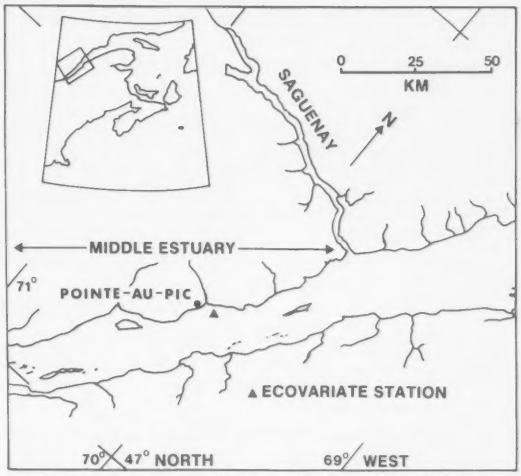


FIG. 1. St. Lawrence Estuary showing station location.

Each time series had 2224 observations (148.3 h). It can be seen (Fig. 2) that the chlorophyll *a* fluorescence record contains a few isolated points that are distinctly greater than the surrounding values. These points may be real but it is likely that they represent an instrument problem and may have an effect on statistical analysis. Therefore, it was decided to remove them from the fluorescence record.

Auto- and cross-spectra were computed using the Fast Fourier Transform algorithm (Cooley and Tukey 1965). Then, coherence and phase spectra were derived from the cross- and auto-spectra. The variance spectra were calculated by dividing a record into subseries of 128 points (8.5

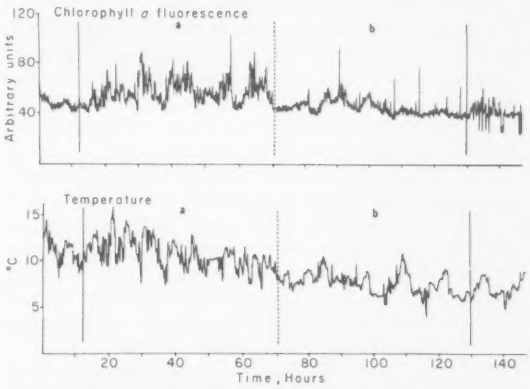


FIG. 2. Time series of chlorophyll *a* fluorescence and temperature. Dashed lines indicate where the series were divided. Sampling interval 4 min.

TABLE 1. Mean and standard deviation of the variables observed during the 119.5-h run.

Variable	First half mean (SD)	Second half mean (SD)
Chlorophyll *a* fluorescence (arbitrary units)	58.07 (9.3)	46.14 (5.4)
Temperature (°C)	10.7 (7.3)	7.6 (5.2)
Temperature gradient (°C/1–10 m)	−0.61 (1.24)	−0.43 (0.67)
Phytoplankton cell counts (cells/L)	19 000 (10 085)	25 900 (9764)
Primary production (mg C/m³/h)	2.9 (1.6)	1.0 (0.7)

h). Each subseries was linearly detrended and its spectrum computed, then the subspectra were averaged. The subseries length was chosen so as to be long enough to preserve sufficient frequency resolution, and short enough to give a reasonable smoothing of the spectral estimates.

Results

There is a marked difference between the first part and the second part of the chlorophyll *a* fluorescence series (Fig. 2). For analysis the first 12.2 h and the last 16.6 h of the series were removed, as the nature of these segments was very different from that of the first and the last part, respectively, of the fluorescence series. The series were divided into two parts of equal length and compared statistically. The mean values of each variable (Table 1) were significantly greater in the first part ($P < 0.001$, *t*-test for means, *F*-test for variances).

The spectrum computed for the first part of the chlorophyll record was quite different from that calculated for the second part. The variance spectra of each part, for both parameters, are presented in Fig. 3. Visually, the spectra of temperature for both parts of the series display the same general pattern (Fig. 3b and d), even if the mean values are significantly different (Table 1). This indicates that the distribution of temperature was controlled by the same factors in both parts. The spectrum of fluorescence in the first part was generally similar to that of temperature (Fig. 3a and b), which suggests that during this period the chlorophyll distribution was probably controlled by processes similar to those controlling temperature. On the other hand, the fluorescence spectra of the second part shows a general pattern markedly different from that of temperature (Fig. 3c and d), and it was therefore felt that the distribution of chlorophyll was controlled by factors other than those controlling temperature.

For statistical comparisons, each spectrum was divided in two equal halves of 32 points: a decreasing slope over the first half ($0.12 < F < 3.75$ cycles·h^{-1}) and a flat second half ($3.87 < F < 7.50$ cycles·h^{-1}).

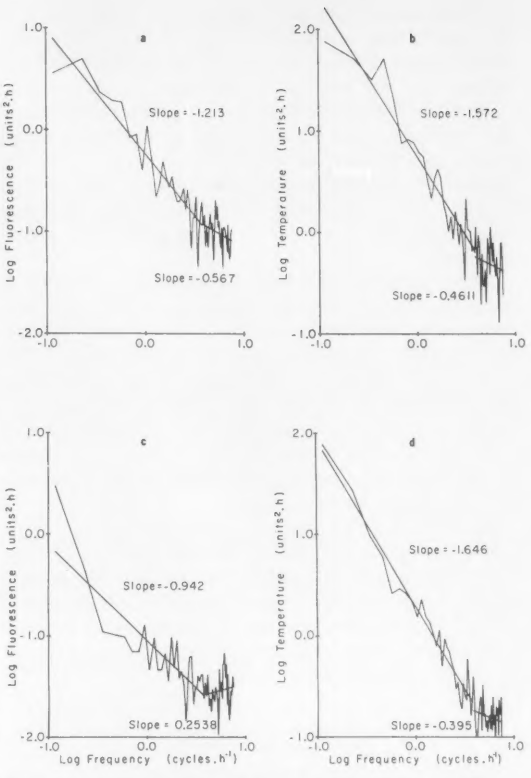


FIG. 3. Spectral density function of chlorophyll *a* fluorescence and temperature for the first (a, b) and the second (c, d) half of the sampling program.

Regression lines were fitted on each half of each spectrum (Fig. 3). For all four spectra, the slopes of the second halves were not significantly different from zero ($P > 0.05$). Therefore, this half of the spectra was associated with random processes and this also indicates that the series were probably not subjected to aliasing. Consequently, the analysis was concentrated on the first half of the spectra. The difference between slopes of the two parameters for frequencies between 0.12 and 3.75 cycles·h^{-1} were assessed for significance. The difference is much smaller for the first part of the series ($t = 2.82$, $0.01 > P > 0.001$) than for the second ($t = 5.72$, $P < 0.001$). The squared coherency (Fig. 4), calculated over the frequencies 0.12–3.75 cycles·h^{-1}, confirms these results. In the first part, the values were significant in five or six frequency bands, while in the second part of the series, the squared coherencies show only one significant value.

Discussion

At an anchor station in an estuarine environment, one observes variations that reflect spatial changes advected

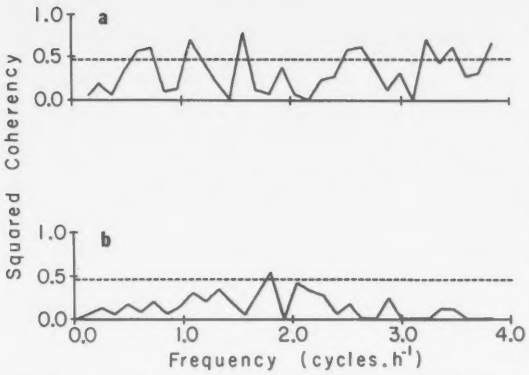


FIG. 4. Squared coherency between chlorophyll *a* fluorescence and temperature spectra for the first (a) and the second (b) part of the study. Dashed lines represent the 95% level of significance when coherence estimates differ from what is expected between two random series (Benignus 1969).

past the station by the periodic movement of water masses. In the present study, it was not possible to get a salinity series comparable to the temperature record, in order to characterize the water masses in terms of both variables. Nevertheless, the current meter provided temperature and salinity data for the first 76 h of the program. The highly significant ($P < .001$) negative correlation (-0.85) between these two factors means that either parameter can be used to describe the water masses. Thus, the primary temperature series is a reliable indicator of water masses present during the sampling period.

The first half of the series coincides with the end of neap tides, and the second half with the beginning of spring tides. At neap tides, there are higher temperatures (and lower salinities) at all times except at the flood, when colder (and saltier) water flows upward from the Lower Estuary. The mean vertical gradients (evaluated as the difference between temperature at 1 and 10 m, Table 1) are significantly ($P < .001$) stronger during the neap tides than during the spring tides. This indicates that the water column is more stratified during the neap tides. Also, both time series show a larger amount of variance during the first part of the records. This is most likely related to the presence of relatively stronger gradients (both horizontal and vertical) during this period. The second half of the series displays lower temperatures (and higher salinities) than observed during the first. This is due to an upward inflow from the Lower Estuary, through the action of larger tidal excursions and increased vertical mixing. However, temperature is higher at the ebb, when the waters seem to be more typically estuarine. Stratification is less pronounced during the spring tide period, and the decreased variance in both fluorescence and temperature records suggests that the gradients are smoothed out by mixing resulting from the increased energy input in the estuary.

The pattern of chlorophyll concentration appears to be related to water movement at a given place. The similitude between temperature and fluorescence, for the first part of the series, suggests that chlorophyll distribution might be controlled by physical processes, mainly tide-induced turbulence, over frequencies between 0.12 and 3.75 cycles·h^{-1}. For a general idea of the length scales involved, the time scale can be converted into a spatial one, using the mean current speed value of about 2 km/h. This transformation is only approximate and therefore should be used with extreme care especially in view of the important variability of the currents in this region. Denman and Platt (1975) also stated that the hypotheses underlying these scale conversions are questionable when applied to the marine environment. Nevertheless, our results indicate that a physical control over chlorophyll distribution is present at scales between ~1 and 20 km. In Long Island Sound, Lekan and Wilson (1978) reported a control of chlorophyll distribution by physical factors for scales ranging from 5 to 20 km. This agrees with our results for larger scales (5–20 km), but is different for shorter scales (1–5 km), where our results still imply a physical control of chlorophyll distribution.

During the second half of the series, fluorescence and temperature show very different patterns, as indicated by the low coherence between their spectra over frequencies between 0.12 and 3.75 cycles·h^{-1}. The general flatter shape of the chlorophyll spectrum could indicate that nonphysical processes influenced chlorophyll distribution. Indeed, the theoretical study of Denman and Platt (1976) suggested that when chlorophyll distribution is controlled by biological processes, the variance of chlorophyll will be a less decreasing function of wavenumber than will that of a passive scalar contaminant-like temperature. They evaluated a characteristic wavenumber, k_c, at which the effect of phytoplankton growth rate is of the same order as the rate of dissipation of turbulent kinetic energy. Denman and Platt (1976) from dimensional arguments concluded that the chlorophyll spectrum should exhibit a $k^{-5/3}$ power-law relationship at wavenumbers larger than k_c, whereas it will display a k^{-1} relationship at wavenumbers less than k_c. Lekan and Wilson (1978) indicated three different scales involved in the control of chlorophyll distribution: (1) length scales under nutrient control (>20 km); (2) a range of wavenumbers over which chlorophyll remains under physical control (5–20 km), and (3) shorter scales (<5 km) where production of chlorophyll is important. Thus, it is generally agreed that a flattening of the chlorophyll spectrum is primarily related to the growth rate of phytoplankton.

Our record of in vivo chlorophyll *a* fluorescence shows that there is less chlorophyll in the second part of the series than in the first. Also, primary production at 1 m was remarkably lower during the second part

of the series. Unexpectedly, phytoplankton cell counts of samples collected hourly on membrane filters at the same depth show higher densities in the second half (Table 1). This might be related to a significant change in the species composition from the first to the second part of the study. Thus, the new population may have had a different fluorescence–chlorophyll *a* relationship. Indeed, other workers report that there are large variations in this relationship according to the species composition (Strickland 1968; Blasco 1973; Kiefer 1973; Loftus and Seliger 1975). In our study, the fluorescence–chlorophyll *a* relationship, as estimated for each of the two parts by model II regression (Sokal and Rohlf 1969), did not show significantly different slopes ($P > 0.05$). Thus, we concluded that the fluorescence–chlorophyll *a* relationship was the same for both parts of the series. Examination of samples collected for cell counts revealed that the phytoplankton population was dominated by the centric diatom *Thalassiosira pacifica* Gran and Angst throughout the entire study.

During the first part of the series, there appeared to be relatively healthy phytoplankton cells, because higher chlorophyll concentrations, higher primary production values, and lower cells counts were noted. On the other hand, during the second part, there were lower chlorophyll concentrations, lower primary production values, and larger cell counts. Thus, there was less chlorophyll per unit cell in the second than in the first part of the study. This indicates that phytoplankton (or chlorophyll) production might have been impeded during the second part. It is possible that a negative stress such as the action of the increased vertical mixing, that was associated with increased tidal energy, influenced the phytoplankton community. Such a lack of stability in the water column could have reduced the residence time of the phytoplankton in the photic layer, thereby preventing optimal photosynthesis. Indeed, after studying the variations of physical, chemical, and biological variables throughout the estuary, Therriault (1973) stated that a marked instability of the water column implies important inhibition of primary production, even though the nutrients are in nonlimiting concentrations. In such conditions, chlorophyll becomes somewhat randomly distributed among phytoplankters, while the cells dispersion remains still controlled by physical processes. A random component would then be introduced in the chlorophyll spectrum, as indicated by the flatter slope plotted for the second part of the study. These results contrast with the observations of most workers. One factor that may account for this difference is that there were low chlorophyll concentrations (mean value of 0.8 $mg \cdot m^{-3}$) in the present study, compared with other works. For example, the results of Lekan and Wilson (1978) show a mean chlorophyll concentration of 3.9 $mg \cdot m^{-3}$. In the present work, the highest values of chlorophyll *a* and primary production occurred during the end of neap tides. Here chlorophyll distribution was controlled by physical processes. With the onset of the spring tides, chlorophyll concentration and primary production showed a marked decrease and a flattening of the chlorophyll spectrum was observed. Such results suggest that this flattening cannot be related to the significant growth rate, but rather to the poor physiological condition of phytoplankton populations that we observed during the spring tide period.

Thus, in the estuarine environment of the St. Lawrence, the tidal regime appears to play an important role in the control of chlorophyll distribution. Yet longer records (perhaps over an entire fortnightly cycle) are needed to confirm this hypothesis and to further elucidate this typically estuarine phenomenon.

Acknowledgments

We thank Dr Y. Ouellet of the Civil Engineering Department, Laval University, for kindly providing computing facilities and helpful counseling concerning spectral analysis. We are indebted to Dr R. Grant Ingram of McGill University for supplying the current meter data and for his valuable suggestions about physical mechanisms in the estuary. Also, thanks to Dr J. Himmelman for helpful criticism and to Ms L. Roy for drawing the figures. This work was supported by grants to GIROQ from the the National Research Council of Canada and Quebec Department of Education and by NRC grant A-9689 to L.L.

BAINBRIDGE, R. 1957. The size, shape and density of marine phytoplankton concentrations. Biol. Rev. 32: 91–115.

BENIGNUS, V. A. 1969. Estimation of the coherence spectrum and its confidence interval using the Fast Fourier Transform. IEEE Trans. Audio Electroacoust. AU-17(2): 145–150.

BLASCO, D. 1973. Estudio de las variaciones de la relacion fluorescencia *in vivo*/clorifila *a* y su aplicacion en oceanografia. Influencia de la limitacion de diferentes nutrientes, efecto del dia y noche y dependencia de la especie estudiada. Invest. Pesq. 37: 533–556.

COOLEY, J. W., AND J. W. TUKEY. 1965. An algorithm for the machine calculation of complex Fourier series. Math. Comput. 19: 297 p.

DENMAN, K. L. 1976. Covariability of chlorophyll and temperature in the sea. Deep-Sea Res. 23: 539–550.

DENMAN, K. L., AND T. PLATT. 1975. Coherences in the horizontal distributions of phytoplankton and temperature in the upper ocean. Mem. Soc. R. Sci. Liège, Ser. 6, 7: 19–30.

1976. The variance spectrum of phytoplankton in a turbulent ocean. J. Mar. Res. 34: 593–601.

FASHAM, M. J. R., and P. R. PUGH. 1976. Observations on the horizontal coherence of chlorophyll *a* and temperature. Deep-Sea Res. 23: 527–538.

FORTIER, L., L. LEGENDRE, A. CARDINAL, AND C. L. TRUMP. 1978. Variabilité à court terme du phytoplancton de l'estuaire du St-Laurent. Mar. Biol. 46: 349–354.

KIEFER, D. A. 1973. Fluorescence properties of natural phytoplankton populations. Mar. Biol. 22: 263–269.

KOLMOGOROV, A. N. 1941. The local structure of turbulence in an incompressible viscous fluid for very large Reynolds Number. Dokl. Akad. Nauk. SSSR. 30: 299–303. (In Russian)

LEKAN, J. F., AND R. E. WILSON. 1978. Spatial variability of phytoplankton biomass in the surface waters of Long Island. Estuarine Coastal Mar. Sci. 6: 239–251.

LOFTUS, M. E., AND H. H. SELIGER. 1975. Some limitations of the *in vivo* fluorescence technique. Chesapeake Sci. 16: 79–92.

LORENZEN, C. J. 1966. A method for the continuous measurement of *in vivo* chlorophyll concentration. Deep-Sea Res. 13: 223–227.

PLATT, T. 1972. Local phytoplankton abundance and turbulence. Deep-Sea Res. 19: 183–187.

PLATT, T., L. M. DICKIE, AND R. W. TRITES. 1970. Spatial heterogeneity of phytoplankton in a near-shore environment. J. Fish. Res. Board Can. 27: 1453–1473.

POWELL, T. M., P. J. RICHERSON, T. M. DILLON, B. A. AGEE, B. J. DOZIER, D. A. GODDEN, AND L. O. MYRUP. 1975. Spatial scales of current speed and phytoplankton biomass fluctuations in Lake Tahoe. Science 189: 1088–1090.

SOKAL, R. R., AND F. J. ROHLF. 1969. Biometry. W. H. Freeman and Co., San Francisco, Calif. 776 p.

STRICKLAND, J. D. H. 1968. Continuous measurement of *in vivo* chlorophyll: a precautionary note. Deep-Sea Res. 15: 225–227.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1972. A practical handbook of seawater analysis. Bull. Fish. Res. Board Can. 167: 310 p.

THERRIAULT, J. C. 1973. Variations des propriétés physico-chimiques et biologiques d'une zone de mélange de l'estuaire du St-Laurent. Thèse de Maîtrise, Univ. Laval, Québec, Qué., 154 p.

Quantification of Vitellogenin in Atlantic Salmon (*Salmo salar*) Plasma by Radioimmunoassay[1]

D. R. IDLER, S. J. HWANG, AND L. W. CRIM

Marine Sciences Research Laboratory, Memorial University of Newfoundland, St. John's, Nfld. A1C 5S7

IDLER, D. R., S. J. HWANG, AND L. W. CRIM. 1979. Quantification of vitellogenin in Atlantic salmon (*Salmo salar*) plasma by radioimmunoassay. J. Fish. Res. Board Can. 36: 574–578.

An antibody prepared against salmon egg yolk proteins has been used to quantify Atlantic salmon (*Salmo salar*) plasma vitellogenin using radioimmunoassay. A low molecular weight fraction isolated from salmon egg yolk was used for radioiodination and as standard solution because plasma vitellogenin could not be iodinated successfully. Parallelism of the egg yolk standard to displacement given by a fraction isolated from vitellogenic salmon plasma and dilutions of plasma samples allowed the assay to be used to evaluate the state of gonadal development of migrating females several months in advance of spawning and for sexing relatively immature salmon.

Key words: salmon, plasma vitellogenin; vitellogenin, quantification in salmon plasma; radioimmunoassay, salmon vitellogenin

IDLER, D. R., S. J. HWANG, AND L. W. CRIM. 1979. Quantification of vitellogenin in Atlantic salmon (*Salmo salar*) plasma by radioimmunoassay. J. Fish. Res. Board Can. 36: 574–578.

Un anticorps produit contre du vitellus d'oeufs de saumon a servi à quantifier la vitellogénine sérique des saumons atlantiques (*Salmo salar*) par dosage radioimmunologique. Une fraction de vitellus de poids moléculaire peu élevé a été marquée à l'iode et a été utilisée comme solution normale car la vitellogénine sérique n'a pu être marquée avec succès. Des dilutions d'une fraction isolée du sérum vitellogénique des saumons et des dilutions de sérum de saumon ont donné des courbes parallèles à la solution normale. Ces résultats nous ont permis de doser des échantillons afin d'évaluer le stade de maturation sexuelle des femelles migratrices plusieurs mois avant la fraie et de déterminer le sexe de saumons relativement immatures.

Received August 29, 1978
Accepted January 22, 1979

Reçu le 29 août 1978
Accepté le 22 janvier 1979

VITELLOGENIN (Vg) is a term used to describe the sex-limited protein in the blood of sexually maturing vertebrates and invertebrates (Pan et al. 1969). Vitellogenin is produced in the liver and its synthesis is stimulated by estrogen (Ansari et al. 1971).

Vitellogenin in serum has been studied primarily in birds (Bergink and Wallace 1974) and amphibians (Wallace and Bergink 1974), but is less well understood in fish (Emmersen and Petersen 1976). Immunological methods were used to show the presence of the major protein constituents of Atlantic cod (*Gadus morhua*) eggs in the serum of female fish with developing ovaries (Plack et al. 1971). Similarly the three major proteins in the salt-soluble fraction of coho salmon (*Oncorhynchus kisutch*) eggs are present in the plasma of sexually maturing female coho and estrogenized prepuberal coho of both sexes (Markert and Vanstone 1971).

[1]M.S.R.L. Contribution No. 327.

Printed in Canada (J5354)
Imprimé au Canada (J5354)

Vitellogenin in fish serum has been quantified indirectly by determination of alkali-labile phosphorus (Emmersen and Petersen 1976). Recently, a radioimmunoassay was developed to measure vitellogenin in laying hen serum; a negligible cross-reaction was found with vitellogenin from fish (Redshaw and Follett 1976).

The purpose of this study was to develop a sensitive and specific radioimmunoassay (RIA) for measuring vitellogenin in plasma of Atlantic salmon (*Salmo salar*), particularly in the early stages of sexual development.

Materials and Methods

PREPARATION OF ANTIBODY

Ovaries (51 g) from sexually mature landlocked Atlantic salmon were homogenized with 200 mL of buffer containing 0.5 M (mol/L) NaCl, 5 mM Na_4 EDTA (Buffer T) using a Waring Blender at high speed for 1 min; after standing at 4°C overnight the homogenate was centrifuged. The supernatant was dialyzed against several changes of distilled water at 4°C and centrifuged. The crude egg yolk pro-

tein precipitate was collected, freeze-dried, and stored at −73°C.

Crude egg yolk protein was dissolved in Buffer T and emulsified with 3 vol of Freund's complete adjuvant. For the primary injection, 1 mL (2.5 mg protein) of the emulsion was injected deeply into the muscle of all four limbs of New Zealand white male rabbits. Three booster injections into pairs of limbs were made with emulsions of 1:1 Buffer T and Freund's complete adjuvant (1.25 mg protein) given at intervals of 4 wk starting 1 mo after the primary injection. The sites for booster injections alternated between fore and hind limbs.

Blood was collected from the ear veins 2 wk after the final injection. Serum was aliquoted, flushed with N_2, and stored at −73°C. Assay titer of the antibody was determined as the dilution that gave 50% binding of ^{131}I-labeled antigen to antibody.

The cross-reaction of antigen (samples)-antibody was determined using RIA. An immunodiffusion method also was employed to check the cross-reactivity of antibody to the samples (Ouchterlony 1949).

Preparation of Material for Radioiodination

Crude salmon egg yolk protein (300 mg) was dissolved in 10 mL of buffer containing 5 mM citric acid, 30 mM 2-amino-2-methyl-1-propanol (Monol), 0.2 mM dithiothreitol (DTT), and centrifuged to remove insoluble material before gel chromatography. Three Sephadex G-200 fractions were detected (Fig. 1) and protein concentration was determined by a modified Lowry method (Idler et al. 1975). Fraction I (tubes 52–80), II (90–106), and III (109–124) had protein contents of 96.8, 12.0, and 8.7 mg, respectively. Fraction II and III ($F_{2,3}$) were pooled at equal protein concentration since Fraction I did not cross react with the antibody. $F_{2,3}$ was used for iodination and standard solution in the Vg-RIA.

Isolation of Salmon Plasma Vitellogenin

Salmon plasma (6.7 mL) was collected from three sexually mature females and treated with $CaCl_2$ according to Redshaw and Follett (1976) and diluted with 60 mL of distilled water. The mixture was held at 4°C overnight then centrifuged. The precipitate was extracted for 2 h with 10 mL of Buffer T, centrifuged, and the pellet reextracted with 4 mL of Buffer T. After centrifugation the supernatants were pooled to 13.6 mL of P-1 (Fig. 2). However, when $CaCl_2$ and 10 volumes of water were added for purifying salmon plasma Vg in this study, only a small amount of precipitate was formed. Ninety-six percent of the substances cross-reacting with the antibody were in S-1. More complete precipitation occurred when S-1 was dialyzed twice against 2L of 5 mM $CaCl_2$ overnight and centrifuged to recover the precipitate that formed during dialysis. This precipitate was dissolved in Buffer T to yield 14 mL of P-2 containing 92% of the cross-reacting material.

Purification of Salmon Plasma Vitellogenin

Ion-exchange chromatography on TEAE-cellulose — TEAE-Cellulose (Cellex T, Bio-Rad, control no. 13232) was sieved and the fraction that remained between 85- and 35-μm sieves was treated according to Wallace (1965). The adsorbent was then suspended and deaerated in 2.5 mM citric acid-15 mM Monol and poured to make a 1.6 × 35 cm column. Fraction P-2 was prepared and chromatographed according to Jared and Wallace (1968) but using a linear gradient (Fig. 3).

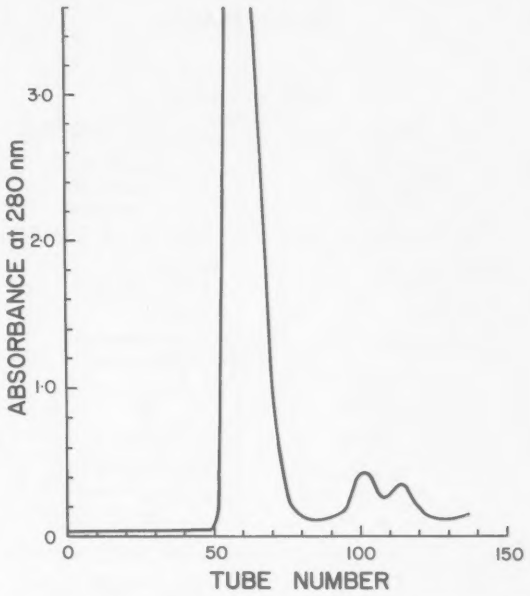


FIG. 1. Chromatography of salmon egg yolk protein on a 2.6 × 95 cm Sephadex G-200 column. Fractions of 4 mL were collected at a flow rate of 15 mL/h and protein was monitored by absorbance at 280 nm.

Gel filtration on Bio-Gel A-0.5 M — The TEAE-cellulose fraction (TEAE-II containing 90% of the immunoreactive material) was dialyzed against Buffer G (0.05 M (mol/L) Tris-Cl, pH 7.7, 0.5 M NaCl, 5 mM Na_4 EDTA, 0.2 mM DTT) and then concentrated to 7 mL using an Amicon PM10 membrane and chromatographed on a 2.6 × 95 cm column of Bio-Gel A-0.5 M (Bio-Rad) with Buffer G. Ninety-six percent of crossreactivity was found in the second peak (Bio Gel-II, Fig. 4).

Phosphorus determination was performed according to Chen et al. (1956).

Radioimmunoassay

Plasma Vg was measured using radioimmunoassay (RIA). The mixture of $F_{2,3}$ was radioiodinated with ^{131}I (Greenwood et al. 1963) and used as the tracer; $F_{2,3}$ also was used as the standard solution for the Vg-RIA.

For radioimmunoassay the antibody was diluted 200 000 times, with a 0.08 M barbital buffer pH 8.6 containing 0.5% bovine serum albumin (BSA) and 0.01% ethylmercurithiosalicylate (Thimerosal). Samples or standard solution (volume for assay 100 μL), antibody (200 μL), and ^{131}I-labeled yolk protein (10 000 cpm/200 μL) were incubated at 4°C for 3 d, and the free and antibody-bound ^{131}I-labeled yolk protein were separated by overnight precipita-

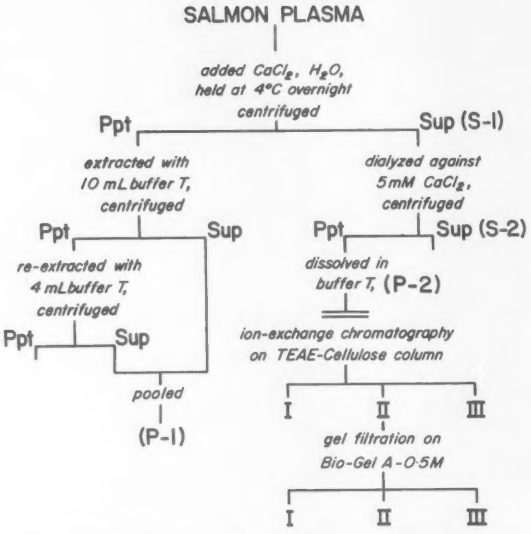


FIG. 2. Isolation and purification of salmon plasma vitellogenin.

tion with normal rabbit serum and goat anti-rabbit γ-globulin. After centrifugation the precipitates were counted in a gamma scintillation spectrometer.

Atlantic salmon blood samples were collected at Greenland and at several locations in Newfoundland. Most extensive sampling was done at Bonavista Bay, Nfld. The sera were stored at −80°C.

Results and Discussion

Though Fraction I (F_1) of gonad yolk was the largest protein peak (82%) off Sephadex G-200 (Fig. 1), it failed to show crossreactivity to the antibody in RIA or immunodiffusion while Fraction II (F_2) and Fraction III (F_3) were immunoreactive. Evidently F_1 was not antigenic to the rabbits in this instance.

When egg yolk $F_{2,3}$ was radioiodinated, and used as the standard solutions for Vg-RIA, the standard curve ranged from 0.05 to 10.0 ng, but the most sensitive and reliable part of the standard curve was between 0.1 and 1.0 ng (Fig. 5). When the logit function is used to linearize the dose–response curve by regression analysis (log concentration vs. logit B/Bo), the slope = −2.59; $R^2 = 0.98$; and ED(50) = 0.32 ng. Eighty-eight percent of labeled $F_{2,3}$ was bound to the excess antibody (5000 × dilution) with a coefficient of variation C.V. = 6.2% (4 assays), and 64% was bound to the 200 000 × diluted antibody (C.V. = 4.2%, $n = 4$). The results revealed that the radioiodination was consistent and suggested $F_{2,3}$ was suitable for labeling for this RIA system. The interassay reproducibility was determined by inserting a salmon plasma sample as the internal standard solution for all assays. The coefficient of variation of the internal standard was 9.5%; $n = 4$.

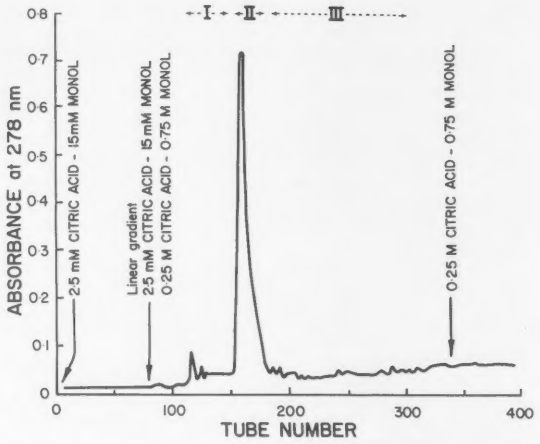


FIG. 3. Ion-exchange chromatography of salmon plasma Vg (P-2) on TEAE-cellulose. Fractions of 4 mL were collected. TEAE-II (tubes 152–178) containing 90% of Vg by radioimmunoassay.

During isolation of the plasma yolk fractions the phosphorus content (% P) increased from 0.65% (P-1 and P-2) to 1.6% for Bio Gel-II, indicative of a progressive enrichment of a fraction corresponding to vitellogenin by its solubility properties and phosphorus content.

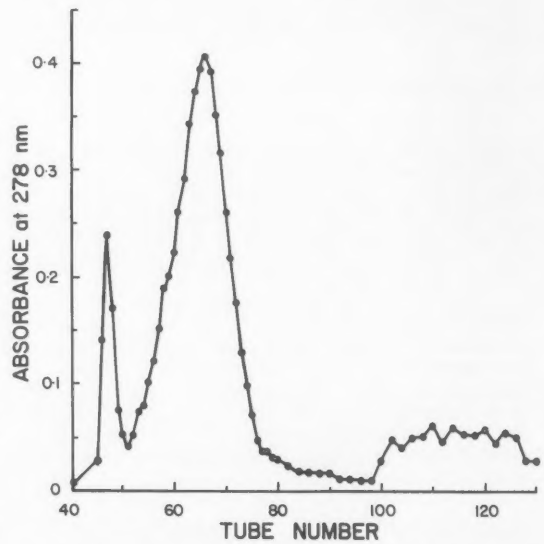


FIG. 4. Elution profile of salmon plasma Vg (TEAE-II) from Bio-Gel A-0.5 M (mol/L). The sample was collected into 4-mL fraction and protein was monitored by absorbance at 278 nm. Bio-Gel-II (tubes 52–76) was the major component with 96% of Vg distributed in this fraction.

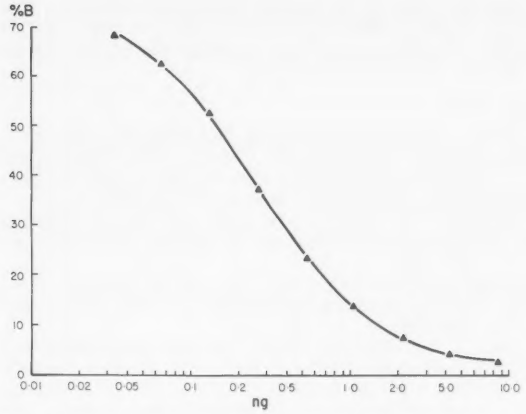


FIG. 5. Standard curve obtained by labeling $F_{2,3}$ and using $F_{2,3}$ as the standard solutions (^{131}I-$F_{2,3}$ vs. $F_{2,3}$).

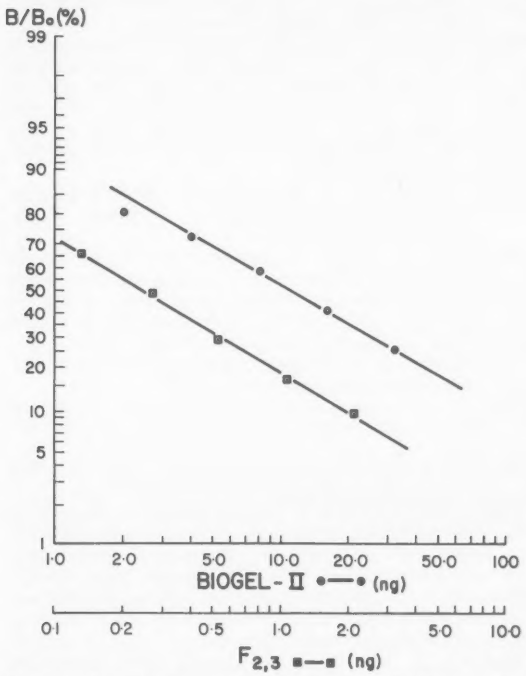


FIG. 6. Parallel displacement curves of ^{131}I-$F_{2,3}$ vs. $F_{2,3}$ system and ^{131}I-$F_{2,3}$ vs. plasma BioGel-II system. The slope $= -2.39$, $R^2 = 0.99$, and ED (50) $= 0.25$ ng when $F_{2,3}$ is used as the standard solution. While the slope $= -2.30$, R^2 0.99, and ED(50) $= 11.99$ ng for the BioGel-II standard curve.

Figure 6 shows the parallel displacement curves obtained when the system ^{131}I-$F_{2,3}$ vs. plasma Bio Gel-II was compared with the routine assay system. Displacement of label requires 47 ± 2 ($n = 3$) times as much Bio-Gel-II as $F_{2,3}$ since there is only a partial recognition of the plasma protein by the anti-yolk antibody. Thus plasma vitellogenin concentrations are 47 times those values obtained in using the routine ^{131}I-$F_{2,3}$ vs. $F_{2,3}$ system. Parallelism of the male (freshwater) and female salmon plasma samples to the standard yolk protein solution has been found by comparison of the dose–response curve for twofold serial dilutions of male samples assayed from 50 to 12.5 μL and female samples assayed from 5×10^{-3} μL to 0.3×10^{-3} μL (Fig. 7).

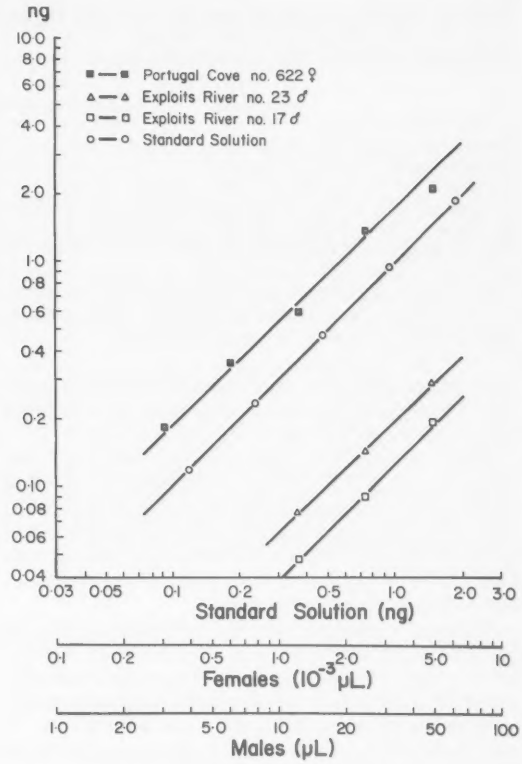


FIG. 7. Dose–response curves for twofold serial dilutions of the egg yolk protein standard solution and Atlantic salmon plasma samples. Parallelism of male and female samples to the standard solution was demonstrated when the male plasma sample was assayed from 50 to 12.5 μL and female sample was diluted and assayed from 5×10^{-3} μL to 0.30×10^{-3} μL. The standard solution ranged between 0.12 and 1.95 ng.

The cross-reactivity of salmon plasma, and the principal component of sera from vitellogenic salmon, to the antibody produced against salmon egg yolk protein enabled the antibody to be employed for testing the salmon plasma Vg.

No Vg was detected in 10 μL of sera from Atlantic

TABLE 1. Mean ± SE (n) vitellogenin content of plasma from female Atlantic salmon from various regions.

	Vg (μg/mL)	Months before spawning[a]
Greenland	5.2±1.9 (59)	14
Bonavista Bay	907±58 (161)	4–5
La Scie	1385±55 (79)	4
St. Anthony	3868±146 (151)	3–4
St. Barbe	3625±306 (67)	3–4
Exploits River	6016±846 (5)	1–2

[a]Times are based on spawning in mid-November; except for Greenland the majority of fish will spawn in the time frame indicated as evidenced by the onset of vitellogenesis.

male salmon from fish taken in Bonavista Bay in June–July. The Vg concentration of sera from female Atlantic salmon are shown in Table 1.

Blood samples from Greenland salmon were collected in August 1974, representing fish 1 yr at sea of which most would likely return to the home coast May–July 1975 for spawning in the fall (~14 mo after capture). Bonavista Bay samples were collected in June 1975. Most of the fish are undergoing spawning migration (Idler et al. unpublished data).

The results noted above reveal that male salmon can be distinguished from females by analysis of a small blood sample. The differences of Vg concentration between the plasma samples from Greenland salmon and samples from Bonavista Bay fish show that the stage of maturity of female salmon may be determined by using Vg-RIA while the fish are still migrating at sea. A relationship between increases in plasma Vg and ovary yolk with advancing maturity of female brown trout were previously reported (Crim and Idler 1978).

No method was previously available for quantifying the small amounts of plasma Vg that occur in female salmon so far in advance of spawning. This radioimmunoassay for plasma Vg is based on immunological cross reactivity between the principal plasma component and yolk proteins.

Labeling of plasma vitellogenin has not proved successful but the ease of labeling and stability of the $F_{2,3}$ preparation facilitates routine assaying and when corrected for the difference in cross-reactivity (X47) give values that correspond to those expected from other researchers (Wallace and Bergink 1974; Emmersen and Petersen 1976).

Acknowledgments

Dr C. M. Campbell provided helpful comments on the manuscript. We thank Mr H. Lear, Fisheries and Environment Canada, for the plasma samples from Greenland fish. This research was supported in part by NRCC Grant A6732 to D.R.I. and a contract with Fisheries and Marine Service, Environment Canada.

ANSARI, A. Q., P. J. DOLPHIN, C. B. LAZIER, K. A. MUNDAY, AND M. AKHTAR. 1971. Chemical composition of an estrogen induced calcium-binding glycolipophosphoprotein in *Xenopus laevis*. Biochem. J. 122: 107–113.

BERGINK, E. W., AND R. A. WALLACE. 1974. Estrogen-induced synthesis of yolk proteins in roosters. Am. Zool. 14: 1177–1193.

CHEN, P. S. JR., T. Y. TORIBARA, AND H. WARNER. 1956. Microdetermination of phosphorus. Anal. Chem. 28: 1756–1758.

CRIM, L. W., AND D. R. IDLER. 1978. Plasma gonadotropin, estradiol and vitellogenin and gonad phosvitin levels in relation to the seasonal reproductive cycles of female brown trout. Ann. Biol. Anim. Biochem. Biophys. 18: 1001–1005.

EMMERSEN, B. K., AND I. M. PETERSEN. 1976. Natural occurrence, and experimental induction by estradiol-17β, of a lipophosphoprotein (vitellogenin) in flounder (*Platichthys flesus*, L.). Comp. Biochem. Physiol. 54B: 443–446.

GREENWOOD, F. C., W. M. HUNTER, AND J. S. GLOVER. 1963. The preparation of ^{131}I-labeled human growth hormones of high specific radioactivity. Biochem. J. 89: 114–123.

IDLER, D. R., S. J. HWANG, AND L. S. BAZAR. 1975. Fish gonadotropin(s). I. Bioassay of salmon gonadotropin(s) *in vitro* with immature trout gonads. Endocrine Res. Comm. 2(3): 199–213.

JARED, D. W., AND R. A. WALLACE. 1968. Comparative chromatography of the yolk proteins of teleosts. Comp. Biochem. Physiol. 24: 437–443.

MARKERT, J. R., AND W. E. VANSTONE. 1971. Egg proteins of coho salmon (*Oncorhynchus kisutch*): chromatographic separation and molecular weights of the major proteins in the high density fraction and their presence in salmon plasma. J. Fish. Res. Board Can. 28: 1853–1856.

OUCHTERLONY, O. 1949. Antigen-antibody reactions in gels. Acta Pathol. Microbiol. Scand. 26: 507.

PAN, M. L., W. J. BELL, AND W. H. TELFER. 1969. Vitellogenic blood protein synthesis by insect fat body. Science 165: 393–394.

PLACK, P. A., D. J. PRITCHARD, AND N. W. FRASER. 1971. Egg protein in cod serum. Biochem. J. 121: 847–856.

REDSHAW, M. R., AND B. K. FOLLETT. 1976. Physiology of egg yolk production by the fowl: the measurement of circulating levels of vitellogenin employing a specific radioimmunoassay. Comp. Biochem. Physiol. 55A: 399–405.

WALLACE, R. A. 1965. Resolution and isolation of avian and amphibian yolk-granule proteins using TEAE-Cellulose. Anal. Biochem. 11: 297–311.

WALLACE, R. A., AND E. W. BERGINK. 1974. Amphibian vitellogenin: properties, hormonal regulation of hepatic synthesis and ovarian uptake, and conversion to yolk proteins. Am. Zool. 14: 1159–1175.

Sorption of Cadmium and its Effect on Growth and the Utilization of Inorganic Carbon and Phosphorus of Two Freshwater Diatoms

H. LEE CONWAY

Ecological Sciences Section, Radiological and Environmental Research Division, Argonne National Laboratory, Argonne, IL 60439, USA

AND SUSAN C. WILLIAMS[1]

Center for Educational Affairs, Argonne National Laboratory, Argonne, IL 60439, USA

CONWAY, H. L., AND S. C. WILLIAMS. 1979. Sorption of cadmium and its effect on growth and the utilization of inorganic carbon and phosphorus of two freshwater diatoms. J. Fish. Res. Board Can. 36: 579–586.

Cadmium was rapidly sorbed by *Asterionella formosa* and *Fragilaria crotonensis* during the first 5–10 min of exposure (0.05 to ~9 μg Cd·L^{-1}), followed by a hyperbolic increase in the cellular cadmium content as a function of time. Results obtained from experiments conducted in the light and dark, experiments using live and dead populations, and cellular site location studies imply that the sorption of cadmium by *A. formosa* is partially an active process, but for *F. crotonensis* it appears to be passive. Populations of *A. formosa* exhibited a corresponding decrease in the growth rate as the ambient cadmium concentration was increased from ~2 to ~9 μg·L^{-1}. In contrast, the growth rate of *F. crotonensis* was unaffected at levels ranging from ~0.05 to ~9 μg Cd·L^{-1}. The utilization of inorganic carbon by *A. formosa* was unaffected at ~4 μg Cd·L^{-1}, but reduced 45% at ~9 μg Cd·L^{-1}. The utilization rates of carbon and phosphorus by *F. crotonensis* were reduced at the lower cadmium concentrations and enhanced at the highest.

Key words: cadmium, toxicity, sorption, diatom, *Asterionella formosa*, *Fragilaria crotonensis*

CONWAY, H. L., AND S. C. WILLIAMS. 1979. Sorption of cadmium and its effect on growth and the utilization of inorganic carbon and phosphorus of two freshwater diatoms. J. Fish. Res. Board Can. 36: 579–586.

Le cadmium est rapidement sorbé par *Asterionella formosa* et *Fragilaria crotonensis* durant les premières 5–10 min d'exposition (0,05 à ~9 μg Cd·L^{-1}). Vient ensuite une augmentation hyperbolique de la teneur en cadmium cellulaire en fonction du temps. Les résultats d'expériences effectuées à la clarté et à la noirceur, d'expériences sur des populations vivantes et mortes, et enfin d'études sur le site d'occurrence dans les cellules laissent penser que la sorption du cadmium par *A. formosa* est en partie un processus actif, alors qu'elle semble passive chez *F. crotonensis*. Si la teneur en cadmium ambiant passe de ~2 à ~9 μg·L^{-1}, les populations de *A. formosa* accusent une diminution correspondante du rythme de croissance. Par contraste, le rythme de croissance de *F. crotonensis* n'est pas affecté par des niveaux allant de ~0,05 à ~9 μg Cd·L^{-1}. L'utilisation du carbone inorganique par *A. formosa* n'est pas affectée à ~4 μg Cd·L^{-1}, mais diminue de 45% à ~9 μg Cd·L^{-1}. Les taux d'utilisation du carbone et du phosphore par *F. crotonensis* diminuent aux faibles teneurs en cadmium et augmentent à la plus haute concentration.

Received July 4, 1978
Accepted February 7, 1979

Reçu le 4 juillet 1978
Accepté le 7 février 1979

CADMIUM ranks as one of the most potentially harmful elements to aquatic ecosystems (Ketchum 1975; Vaughan et al. 1975). It has no known biological function in plants (Fleischer et al. 1974); however, it is rapidly sorbed by phytoplankton and adversely affects growth and photosynthesis (Zingmark 1972; Hutchinson 1973; Klass et al. 1974; Patin et al. 1974; Sick and Windom 1975; Berland et al. 1976; Cossa 1976).

Conway (1978) measured the sorption and effects of cadmium on growth and micronutrient utilization of *Asterionella formosa*. The present study is a continuation of this work with special emphasis on sorption kinetics. Our objective was to determine the short-term sorptive characteristics of cadmium and the impact of cadmium exposure on the rates of growth, photosynthesis, and phosphorus utilization of two ecologically important diatom species of the Great Lakes, *Asterionella formosa* and *Fragilaria crotonensis*.

[1]Undergraduate research participant from St. Olaf's College, Minn.

Printed in Canada (J5294)
Imprimé au Canada (J5294)

Materials and Methods

Experiments were short-term, spanning approximately one generation time of the diatom populations. The sorption and effects experiments were completed under batch-culture conditions; however, the populations used in the experiments had previously been grown in steady-state chemostats, at a dilution rate of $0.02 \cdot h^{-1}$ (generation time of ~35 h), using synthetic lake water. In the present study, the phosphate and silicate concentrations in the synthetic lake water described by Conway (1978) were increased to 0.4 and 25 $\mu mol \cdot L^{-1}$, respectively. The chemostat system and the methods for reactive phosphate analysis and population biovolume were described in Conway (1978). A series of experimental cultures were set up by adding a 1-L portion of a unialgal, steady-state population, diluted with an equal volume of synthetic lake water, to a 2-L pyrex bottle. These cultures were then treated as described below.

The sorption of cadmium by *A. formosa* and *F. crotonensis* populations was studied by adding a solution (≤ 1 mL) of radioactive (^{115}Cd) plus stable cadmium in 0.04 N HCl to the populations in the 2-L bottles. No detectable change in the pH of the culture medium was observed. The specific activity ranged from 0.1 to 2.5 $\mu Ci \cdot \mu g^{-1}$. Duplicate samples for cadmium associated with the phytoplankton cells and in solution were taken at the time of cadmium addition, at 15-min intervals during the 1st h (except in Experiment S3) and at varying times thereafter. Biovolumes were measured, using a Coulter Counter Ta-II, initially and at 2- and 3-h intervals. The sample preparation and scintillator used for ^{115m}Cd were described previously (Conway 1978).

Cadmium sorption was determined for (1) live populations of *A. formosa* or *F. crotonensis* at ambient levels of ~2, 4, and 9 $\mu g\, Cd \cdot L^{-1}$ (Experiment S1), (2) live *F. crotonensis* populations at ~0.05, 0.1, 0.2, and 0.4 $\mu g\, Cd \cdot L^{-1}$ (Experiment S2), (3) live populations of *A. formosa* or *F. crotonensis* in the light or dark at an ambient concentration between 4 and 5 $\mu g\, Cd \cdot L^{-1}$; desorption was studied by resuspending these populations in cadmium-free medium (Experiment S3), and (4) dead populations of *A. formosa* or *F. crotonensis* at an ambient concentration between 4 and 5 $\mu g\, Cd \cdot L^{-1}$; the cells were killed by the addition of $HgCl_2$ (~25 $mg \cdot L^{-1}$) or by freezing with liquid nitrogen and thawing before the start of the experiment (S4). Ultrasonic fractionation, combined with selective filtration and photooxidation, was used to determine the cellular location of cadmium after the 1st and 24th h of cadmium exposure at ~4 $\mu g\, Cd \cdot L^{-1}$ (Experiment S5); duplicate samples were taken for these analyses. The sorption site location procedure has been described previously (Conway 1978). Table 1 summarizes the experimental conditions of the sorption studies.

The effect of cadmium on growth, photosynthesis, and phosphate uptake was determined in a series of experiments at cadmium concentrations similar to those used in the sorption studies. Four culture bottles were prepared with sterile medium and preconditioned diatom populations as described above. Stable cadmium, as $CdCl_2$ in 0.04 N HCl, was added to the two test populations and an equivalent amount of acid to the two controls; no detectable change in pH occurred. Following a 12-h preincubation period with cadmium, ^{14}C or ^{33}P was added to one of the test populations and one of the controls. Biovolumes, particulate and dissolved ^{14}C or ^{33}P, and ambient phosphate concentrations were measured at varying times during the course of the experiment. Radioactive ^{14}C and ^{33}P samples were counted using the procedure described for ^{115m}Cd (Conway 1978). Specific activities ranged from 8 to 23 $\mu Ci \cdot \mu g^{-1}$ for ^{14}C and 10^{-2} to 10^{-1} $\mu Ci \cdot \mu g^{-1}$ for ^{33}P. The concentration of dissolved cadmium, culture conditions, and diatom species used in the effects experiments were the same as those employed for Experiments S1 and S2; however, effects experiments on carbon and phosphorus uptake were not done at ~2 $\mu g\, Cd \cdot L^{-1}$. The ambient concentration of cadmium or inorganic carbon remained essentially constant during the experiments, i.e. initial activity $\approx$ final activity. Inorganic phosphorus was rapidly utilized during the experiments; about 65% of the initial activity remained in solution at the end of the experiment; however, 90–110% of the total activity could be accounted for in solution and associated with particulates.

Growth rates were computed according to the equation

$$x = x_0 e^{\mu t}$$

or

$$\mu = (\ln x - \ln x_0)/t \quad (1)$$

TABLE 1. Summary of experimental conditions for the sorption studies.

Experiment number	Ambient cadmium concn ($\mu g \cdot L^{-1}$)	Condition of cells	Duration of experiment (h)	Light or dark incubation	Species
S1	2, 4, 9	live	~24	light	*A. formosa* *F. crotonensis*
S2	0.05, 0.1, 0.2 and 0.4	live	~24	light	*F. crotonensis*
S3	4–5	live	~24[a]	light, dark	*A. formosa* *F. crotonensis*
S4	4–5	Hg-killed cold-killed	~7	light	*A. formosa* *F. crotonensis*
S5	~4	live	24	light	*A. formosa* *F. crotonensis*

[a]Desorption portion lasted an additional 52 h.

TABLE 2. Coefficient of variation (C.V.) for sampling procedure and resulting analyses of ^{14}C, ^{33}P, and ^{115m}Cd determined at four time periods during the course of an experiment. Six replicates were taken at each time period. n.a. = not available.

Isotope	Hours after isotope addition	C.V. filtrate	C.V. particulate
^{14}C[a]	2	2	n.a.
	8	2	1
	12	1	2
	22	1	3
^{33}Pb	3	1	4
	8	1	4
	13	2	1
	22	1	3
^{115m}Cd[c]	1	2	6
	7	2	6
	12	1	6
	25	1	3

[a]Filtrate units = mg C·L^{-1}; particulate units = μg C·mm^{-3}.
[b]Filtrate units = μmol P·L^{-1}; particulate units = μmol P·mm^{-3}.
[c]Filtrate units = μg Cd·L^{-1}; particulate units = ng Cd·mm^{-3}.

where μ is the growth rate (h^{-1}), x_0 is the biovolume (mm^3·L^{-1}) at time zero, and x is the biovolume at time t.

The radioactive ^{14}C and ^{33}P associated with the diatom cells was expressed as micrograms of inorganic carbon (C) or phosphorus (P) per cubic millimetre of cell biovolume. The utilization rate of inorganic carbon or phosphorus included the nonradioactive (stable) ion. The initial concentration of stable inorganic carbon was obtained from alkalinity measurements (Golterman and Clymo 1971); stable inorganic phosphorus was determined as reactive phosphate using an autoanalyzer. The uptake rate (V_c; using carbon as an example) was calculated from the equation

$$V_c = \Delta c/(\bar{x}\cdot\Delta t), \tag{2}$$

where Δc is the change in cellular carbon (μg C) and $\bar{x}$ the average value of population biovolume (mm^3) during the time interval Δt (h). The resulting units are micrograms of carbon (mm^3·h)$^{-1}$.

Statistical variation in the sampling procedure and resulting radioactive measurements was determined by duplicating the sorption and effects experiments at representative concentrations (and activities) of cadmium, carbon, and phosphorus. Six replicate samples were taken at four time periods during the course of an experiment (Table 2). The same coefficient of variation was obtained using either *A. formosa* or *F. crotonensis*. The coefficient of variation for biovolume determination was 5% using 10 replicates.

The cellular contents of cadmium, carbon, and phosphorus were normalized to cell volume in this study. However, values of amorphous silica, chlorophyll *a*, and organic carbon, obtained from steady-state, chemostat populations and normalized to cell volume, were not significantly different between the two species. Therefore bias was not introduced into the calculations of cadmium, carbon, and phosphorus utilization by normalizing to cell volume instead of an organic biomass parameter.

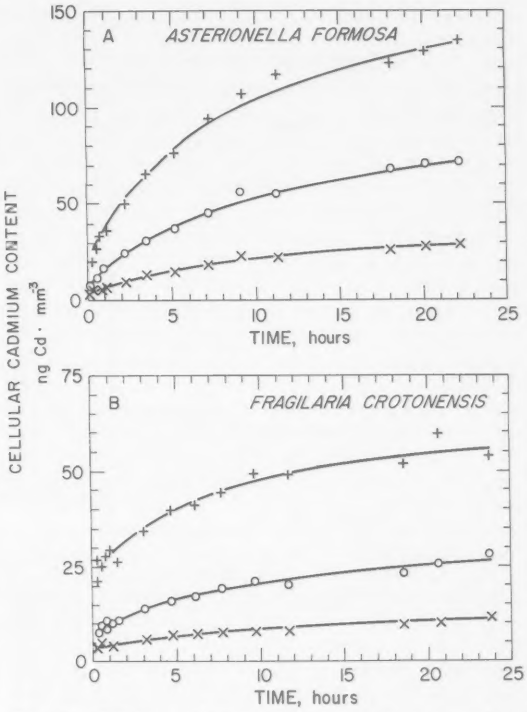


FIG. 1. Cellular cadmium content vs. time at ambient cadmium concentrations of ~2 μg·L^{-1} (×), ~4 μg·L^{-1} (○), and ~9 μg·L^{-1} (+), Experiment S1. A hyperbolic curve has been fitted to the data points.

Results

SORPTION

The time-dependent sorption of cadmium by *A. formosa* and *F. crotonensis* populations was a hyperbolic function (Fig. 1) described by the equation

$$S - S' = \frac{(S_{max} - S')t}{K + t} \tag{3}$$

where S is the cellular cadmium content (ng Cd·mm^{-3}) at time t, S_{max} is the maximum cellular cadmium content, K is a constant equal to the time to reach $S_{max}/2$, and S' is the initial rapid sorption of cadmium at $t \rightarrow 0$. Both species rapidly sorbed cadmium (Fig. 1) as represented by the initial data point taken during the first 5–10 min of the experiment. The S' values for both species at 2, 4, and 9 μg Cd·L^{-1} were similar at each respective cadmium concentration; however, values of S_{max} for *A. formosa* populations were ~3 times larger than those observed for *F. crotonensis* (Table 3).

TABLE 3. Values of S', S_{max}, and bioaccumulation ratio (BR) at different ambient cadmium concentrations in Experiments S1 and S2.

Species	Ambient cadmium concn ($\mu g \cdot L^{-1}$)	S' (ng Cd·mm^{-3})	S_{max}[a] (ng Cd·mm^{-3})	BR[b,c]
Asterionella formosa	1.9	2.5	43 (2)	22 600
	4.1	7.0	100 (4)	24 400
	8.8	17.0	179 (9)	20 300
Fragilaria crotonensis	0.05	0.2	0.3 (0.05)	6 000
	0.1	0.5	0.6 (0.08)	6 000
	0.2	0.3	0.7 (0.1)	3 500
	0.4	1.5	1.7 (0.4)	4 000
	1.8	2.4	12 (1)	6 600
	4.5	7.0	36 (2)	8 000
	8.5	18.0	64 (3)	7 500

[a]Standard deviation is in parentheses and was calculated using the statistical methodology of Cleland (1967).

[b]Defined as (concentration of Cd in/on the organism)·(concentration of Cd in the medium)$^{-1}$.

[c]These ratios were calculated using the ambient cadmium concentrations and S_{max} values in this table.

In Experiment S3, the initial rapid sorption of cadmium (S') in the dark was comparable to that observed in the light (Fig. 2). The cellular cadmium content of the *A. formosa* population in the light, just before resuspending the cells in cadmium-free medium, was approximately twice that observed in the dark; however, the cellular cadmium content of both *F. crotonensis* populations was approximately the same. During the 1st 2 h in cadmium-free medium, the cellular cadmium content decreased to ~45% of the value just before resuspension for *A. formosa* and ~28% for *F. crotonensis*; at the end of 52 h in the cadmium-free medium this percentage was ~5% for both species (Fig. 2).

In Experiment S4, the initial rapid sorption of cadmium (S') by the cold-killed populations was ~2 (*F. crotonensis*) to 4 (*A. formosa*) times higher than that observed for the respective live populations (Table 4). The parameters S_{max} and bioaccumulation ratio (BR), for either the Hg-killed or the cold-killed population, were approximately the same for both species.

The results of the cell fractionation study (Experiment S5) indicate that after the 1st h of cadmium exposure there was a greater percentage of the total cellular cadmium content associated with the cell contents of *F. crotonensis* (56) than for the cell contents of *A. formosa* (37). At the end of 24 h this percentage had increased to 74 for *A. formosa* while remaining essentially constant for *F. crotonensis*. The remainder of the cellular cadmium was associated with the organic coating of the frustule; cadmium was not found in the frustule at either time period.

EFFECTS

A reduction in the growth rate of *F. crotonensis* was not observed in the range of 0.05 to ~9 μg Cd·L^{-1} (Table 5); however, *A. formosa exhibited* 18, 25, and

TABLE 4. Values of S', S_{max}, and bioaccumulation ratio (BR) for live, cold-killed and Hg-killed populations at an ambient cadmium concentration between 4 and 5 $\mu g \cdot L^{-1}$ for Experiment S4.

Species	Condition of population	S' (ng Cd·mm^{-3})	S_{max}[a] (ng Cd·mm^{-3})	BR
Asterionella formosa	live	7	100 (4)	24 400
	cold-killed	31	43 (2)	11 000
	Hg-killed	3	9 (3)	2 100
Fragilaria crotonensis	live	7	36 (2)	8 000
	cold-killed	16	43 (2)	9 000
	Hg-killed	3	11 (1)	2 200

[a]Standard deviation is in parentheses and was calculated using the statistical methodology of Cleland (1967).

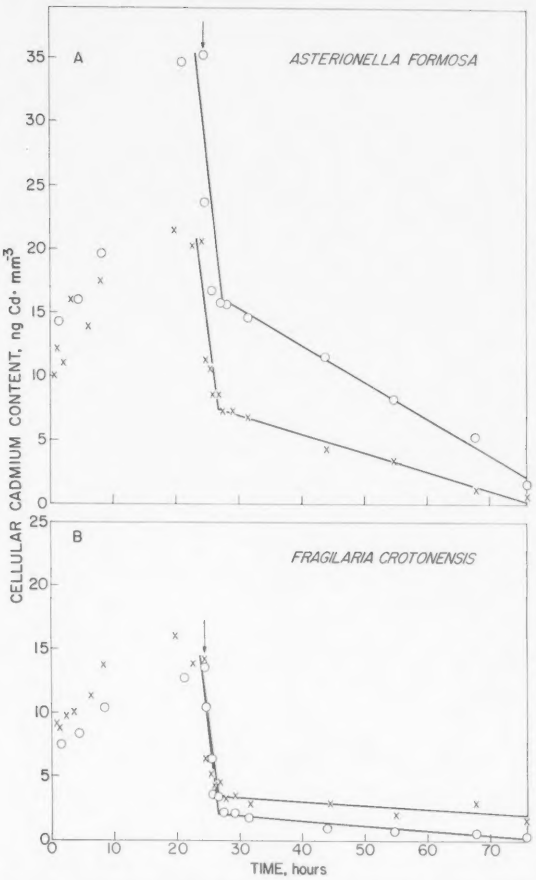


FIG. 2. Cellular cadmium content vs. time in the light (○) and dark (×), Experiment S3. Arrow (↓) indicates point at which cells were resuspended in cadmium-free medium. Straight lines were fitted to the data points after the arrow for the 1st 2 and the final 50 h in cadmium-free medium.

46% reductions in the growth rate at ~2, ~4, and ~9 μg Cd·L^{-1}, respectively. The utilization of inorganic carbon by *A. formosa* was not affected at ~4 μg Cd·L^{-1}, but it was reduced by 45% at ~9 μg Cd·L^{-1}. The utilization rates of carbon and phosphorus by *F. crotonensis* were reduced at the lower cadmium concentrations (<0.5 μg Cd·L^{-1}) and enhanced at the highest (~9 μg Cd·L^{-1}).

Discussion

Sorption

The initial rapid sorption (S') observed during the first 5–10 min of cadmium exposure was characteristic of both species at all ambient cadmium concentrations used in this study and was similar to that observed by other workers for this and other elements (e.g. Rice and Willis 1959; Mandelli 1969; Sick and Windom 1975). At the end of the 1st h of cadmium exposure a large fraction of cellular cadmium was found on the organic coating of the cell wall and in the cell contents, implying that S' is a mixture of adsorptive and absorptive processes. The sulfated polysaccharides found in the organic coating of the diatom wall (Nakajima and Volcani 1969) may function as an ion exchange resin (Percival and MacDowell 1967). This feature could account for the large percentage of cadmium presumably adsorbed on the organic coating of the frustule. The rapid penetration of cadmium into the cell may be attributed to its chemical similarities to zinc which is required as a coenzyme in metabolic processes (Vallee and Ulmer 1972). The different S' values observed between the cold-killed and Hg-killed populations can be explained as competition between cadmium and mercury for binding sites. Since the mercury was present at ambient molar concentrations ~10^3 times higher than cadmium, it is not surprising that S' values of Hg-killed populations would be much lower than the cold-killed populations. The difference in S' values between live and cold-killed populations, however, are more difficult to explain. The disruption of cellular metabolic processes in the cold-killed populations apparently resulted in a greater number of initial binding sites than were found in the live populations; however, the explanation for this difference is not readily apparent.

In contrast to the S' value, the maximal cellular cadmium content of the live *A. formosa* populations was ~3 times larger than that observed for *F. crotonensis*. Also, the ratio S_{max} (live)/S_{max} (cold-killed) was >2 for *A. formosa* and <1 for *F. crotonensis*, and the ratio of cadmium sorption in the light to that in the dark was ~2 for *A. formosa* but only ~1 for *F. crotonensis*. These observations, together with the data showing the increase in cadmium associated with the cell contents of *A. formosa* from the 1st to the 24th h of cadmium exposure, support the idea that some cadmium is actively sorbed by *A. formosa* while for *F. crotonensis* it appears that the predominant sorption mechanism is passive.

The lower S_{max} values of *F. crotonensis*, compared to *A. formosa*, may have been the result of the passive sorption characteristics of this species and simultaneous excretion or desorption of cadmium back into the medium. Although S_{max} values increased with increasing ambient levels of cadmium, and the BR was approximately the same for each (live) species at the various cadmium levels, i.e. ~22 000 for *A. formosa* and ~7000 for *F. crotonensis* (except at 0.2 and 0.4 μg Cd·L^{-1}), indicating that a saturation of active and passive sites had occurred. At an ambient cadmium concentration of ~10 μg Cd·L^{-1}, Conway (1978) found a BR of ~40 000 in long-term studies with *A.*

TABLE 5. Percent reduction in growth rate and carbon and phosphorus utilization during a 24- or 36-h exposure to cadmium. n.a. = not available.

Species	Ambient cadmium concn ($\mu g \cdot L^{-1}$)	Growth rate, μ[a] (h^{-1})	Carbon uptake rate, V_c[a] $\mu g\ C \cdot (mm^3 \cdot h)^{-1}$	Phosphorus uptake rate, V_p[a,b] $\mu g\ P \cdot (mm^3 \cdot h)^{-1}$
Asterionella formosa	1.9	18%	n.a.	n.a.
	4.1	25%	no effect[c]	n.a.
	8.8	46%	45%	n.a.
Fragilaria crotonensis	0.05	no effect[c]	no effect[c]	7%
	0.1	no effect[c]	48%	19%
	0.2	no effect[c]	52%	5%
	0.4	no effect[c]	46%	16%
	1.8	no effect[c]	n.a.	n.a.
	4.5	no effect[c]	20%	−10%[d]
	8.5	no effect[c]	−6%[d]	−18%[d]

[a]Percent reduction in the rates of uptake or growth was calculated from the equation: % reduction = [(control value − test value)/(control value] × 100.

[b]These percentages were calculated for the last 12 h of a 24-h exposure to cadmium.

[c]No effect if percent increase or decrease was less than the coefficient of variation for that parameter (see Table 2 and text).

[d]Negative values indicate that the test population increased uptake in the presence of cadmium.

formosa while Cossa (1976) found a BR of ~10 000 for *Phaeodactylum tricornutum*, a marine diatom.

The rapid desorption of cadmium, observed during the initial 2 h in cadmium-free medium, indicates a "loosely-bound" fraction readily released by the cell. This fraction may be the cadmium adsorbed by the cell and if so indicates that a greater percentage of adsorbed cadmium is characteristic of *F. crotonensis* (~70) than for *A. formosa* (~55). After the initial desorption, the rate decreased 10–100 times and may represent the rate of cadmium excreted from the cell contents. Sorption and desorption may occur simultaneously, in which case the desorption could only be observed when the sorption process was stopped. The residual cellular cadmium at the end of this experiment (~2 ng $Cd \cdot mm^{-3}$) has also been observed as a constant value at the end of ~2 mo in cadmium-free medium (unpublished data) and may represent that portion irreversibly tied up in metalloenzymes and complexed with amino acids, peptides, and proteins. It is therefore conceivable that <5% of the total cadmium sorbed by the cell is actively involved in the detrimental effects observed in the test populations.

EFFECTS

The growth rate of *A. formosa* was profoundly affected by cadmium exposure, in contrast to *F. crotonensis*. Furthermore, the reduction in growth rate was greater at the higher ambient cadmium concentrations, i.e. at the higher cellular cadmium content. The fact that *F. crotonensis* had ~3 times less cellular cadmium does not totally account for its unaffected growth rate since S_{max} values overlapped between the two species. Passive sorption of cadmium by *F. crotonensis*, which may have resulted in lower levels of cadmium associated with the cell contents, could have been an important mechanism preventing the cadmium from adversely affecting the growth rate.

Comparing our results with those of Conway (1978), we see a more pronounced reduction in the growth rate with increased exposure time to cadmium, at corresponding cellular cadmium and ambient cadmium concentrations. This would strengthen the concept that the duration of exposure to cadmium plays an important role in reducing the growth rate of the cell. Cadmium not only affects a large number of metabolic enzyme systems by replacing zinc as the coenzyme, but it also fractionates the total RNA of the bacterium *Escherichia coli* and markedly changes the physical properties of DNA (Vallee and Ulmer 1972). The time-dependent effect of cadmium may be a reflection of its detrimental characteristics during synthesis of RNA and DNA since these metabolic controllers require a finite time to be seriously affected.

The utilization of inorganic carbon and phosphorus by *F. crotonensis* was affected by exposure to cadmium, in contrast to the lack of any measurable effect on the growth rate. The dissimilar response in the utilization of carbon and phosphorus, relative to growth during cadmium exposure, may be the result of uncoupling between uptake and growth, which has been observed in freshwater and marine phytoplankton (e.g. Healey and Hendzel 1975; Morris et al. 1974; Perry 1976). The effect of cadmium on the utilization of carbon and phosphorus was similar for both elements, i.e. a substantial reduction at the lower cadmium concentrations (<0.5 $\mu g\ Cd \cdot L^{-1}$) which changed to enhanced utilization at the highest ~9 $\mu g\ Cd \cdot L^{-1}$). The reduction in phosphorus utilization at low cadmium concentrations may have been the result of substitution of cadmium for zinc in alkaline phosphatase, thereby par-

tially inactivating the enzyme's function. This has been shown to occur in the bacterium *E. coli* (Applebury et al. 1970). The enhanced utilization of phosphorus (and carbon) at higher cadmium concentrations may indicate a breakdown in the feedback control of the permease system transporting phosphorus (or carbon) into the cell; similar to the mechanism postulated for the inhibition of nitrate uptake by ammonium (Conway 1977) and the initial surge in micronutrient utilization by nutrient-limited populations (Rhee 1973; Conway et al. 1976). The reduction in the utilization of carbon and phosphorus, and the disruptive consequences of their enhanced utilization, implies a time-dependent effect on growth. That is, the growth rate could not remain unaffected at a reduced or enhanced rate of carbon and phosphorus utilization. Conway (1978) found an enhanced utilization of silicon and phosphorus before cessation of micronutrient utilization and growth. This would support the contention that given sufficient exposure time to cadmium the growth rate of *F. crotonensis* would have been reduced; however, this requires verification.

Implications

In this study we have demonstrated a dissimilar physiological response of two ecologically important diatom species to cadmium. The different sorptive characteristics of cadmium by the two species have been pointed out as a probable mechanism by which phytoplankton in the same class can have very different physiological responses to the same pollutant under similar environmental and metabolic conditions. The results of this study, together with those of Conway (1978), have shown that increasing the exposure time to cadmium can lead to more pronounced detrimental effects on phytoplankton physiology. Chronic, long-term effects, resulting from cadmium exposure together with dissimilar physiological responses among species, could conceivably lead to changes in species diversity in aquatic systems. Extrapolation of the results of a short-term study to the natural environment has many inherent problems, as pointed out by Stockner and Antia (1976). However, the short-term study remains a powerful tool in the screening of potentially harmful compounds and the examination of their possible causative mechanisms.

Acknowledgments

We thank Dr W. F. Danforth (Illinois Institute of Technology, Chicago) for the inocula of *Asteriolla formosa* and *Fragilaria crotonensis*, and Drs D. Edgington, D. Mellinger, and J. Parker for their comments on the manuscript. Work performed under the auspices of the U.S. Department of Energy.

Applebury, M. E., B. P. Johnsen, and J. E. Coleman. 1970. Phosphate binding to alkaline phosphatase. J. Biol. Chem. 245: 4968–4975.

Berland, B. R., D. J. Bonin, V. I. Kapkov, S. Y. Maestrini, and D. P. Arlhoc. 1976. Toxic action of four heavy metals upon the growth and unicellular marine algae. C. R. Acad. Sci. Ser. D 282: 633–636.

Cleland, W. 1967. The statistical analysis of enzyme kinetic data. Adv. Enzymol. 29: 1–32.

Conway, H. L. 1977. Interactions of inorganic nitrogen in the uptake and assimilation by marine phytoplankton. Mar. Biol. 39: 221–232.

1978. Sorption of arsenic and cadmium and their effects on growth, micronutrient utilization, and photosynthetic pigment composition of *Asterionella formosa*. J. Fish. Res. Board Can. 35: 286–294.

Conway, H. L., P. J. Harrison, and C. O. Davis. 1976. Marine diatoms grown in chemostats under silicate or ammonium limitation. II. Transient response of *Skeletonema costatum* to a single addition of the limiting nutrient. Mar. Biol. 35: 187–199.

Cossa, D. 1976. Sorption of cadmium by a population of the diatom *Phaeodactulum tricornutum* in culture. Mar. Biol. 34: 163–167.

Fleischer, M., A. F. Sarofim, D. W. Fassett, P. Hammond, H. T. Schacklette, I. C. T. Nisbet, and S. Epstein. 1974. Environmental impact of cadmium: a review by the panel on hazardous trace substances. Environ. Health Perspect. 1974: 253–323.

Golterman, H. L., and R. S. Clymo [ed.]. 1971. Methods for chemical analysis of freshwaters. IBP Handbook No. 8. Blackwell, Oxford. 166 p.

Healey, F. P., and L. L. Hendzel. 1975. Effect of phosphorus deficiency on two algae growing in chemostats. J. Phycol. 11: 303–309.

Hutchinson, T. C. 1973. Comparative studies on the toxicity of heavy metals to phytoplankton and their synergistic interactions. Water Poll. Res. Can. 7: 68–90.

Ketchum, B. H. 1975. Problems in aquatic ecosystems, with special reference to heavy metal pollution of the marine environment, p. 76–82. *In* A. D. McIntyre and C. F. Mills [ed.] Ecological toxicology research: effects of heavy metal and organohalogen compounds, Vol. 7. Plenum Press, New York, N.Y.

Klass, E., D. W. Rowe, and E. J. Massaro. 1974. The effect of cadmium on population growth of the green alga, *Scenedesmus quadracauda*. Bull. Environ. Contam. Toxicol. 12: 442–445.

Mandelli, E. F. 1969. The inhibitory effects of copper on marine phytoplankton. Mar. Sci. 14: 38–41.

Morris, I., H. E. Glover, and S. C. Yentsch. 1974. Products of photosynthesis by marine phytoplankton: the effects of environmental factors on the relative rates of protein synthesis. Mar. Biol. 27: 1–9.

Nakajima, T., and B. E. Volcani. 1969. 3,4-dihydroxyproline: a new amino acid in diatom cell walls. Science 164: 1400–1401.

Patin, S. A., V. N. Tkachenko, A. M. Ibragim, and L. V. Fedotova. 1974. Effect of some metals on primary production in the coastal zone of the Caspian Sea. Okeanologiya 14: 72–74.

Percival, E., and R. H. McDowell. 1967. Chemistry and enzymology of marine algal polysaccharides. Academic Press, New York and London.

Perry, M. J. 1976. Phosphate utilization by an oceanic diatom in phosphorus limited chemostat culture and in the oligotrophic waters of the central north Pacific. Limnol. Oceanogr. 21: 88–107.

Rhee, G-Y. 1973. A continuous culture study of phosphate

uptake, growth rate and polyphosphate in *Scenedesmus* sp. J. Phycol. 9: 495–506.

RICE, T. R., AND V. M. WILLIS. 1959. Uptake, accumulation and loss of radioactive cesium-144 by marine planktonic algae. Limnol. Oceanogr. 4: 277–290.

SICK, L. V., AND H. L. WINDOM. 1975. Effects of environmental levels of mercury and cadmium on rates of metal uptake and growth physiology of selected genera of marine phytoplankton, p. 239–249. *In* F. G. Howell et al. [ed.] Mineral cycling in southwestern ecosystems. Technical Information Center, U.S. Energy Research and Development Administration.

STOCKNER, J. G., AND N. J. ANTIA. 1976. Phytoplankton adaptation to environmental stresses from toxicants, nutrients and pollutants—a warning. J. Fish. Res. Board Can. 33: 2089–2096.

VALLEE, B. L., AND D. D. ULMER. 1972. Biochemical effects of mercury, cadmium and lead, p. 91–128. *In* E. E. Snell et al. [ed.] Annual review of biochemistry, Vol. 4. Annual Reviews, Inc.

VAUGHAN, B. E., K. H. ABEL, D. A. CATALOLO, J. M. HALES, C. E. HANE, L. A. RANCIETELLI, R. C. ROUTSON, R. E. WILDUNG, AND E. G. WOLFE. 1975. Review of potential impact on health and environmental quality from metals entering the environment as a result of coal utilization. A Battelle Energy Program Report. Battelle Memorial Institute, Richland, Wash. 75 p.

ZINGMARK, R. G. 1972. Effect of mercury and cadmium on the photosynthetic rates of Atlantic oceanic and estuarine phytoplankton populations. J. Phycol. (Suppl.) 8: 9.

Pathogenesis of Infectious Pancreatic Necrosis in Atlantic Salmon (*Salmo salar*)

ROBERT N. SWANSON AND JAMES H. GILLESPIE

Department of Microbiology, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA

SWANSON, R. N., AND J. H. GILLESPIE. 1979. Pathogenesis of infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 36: 587–591.

Atlantic salmon (*Salmo salar*) fry and yearling were found to undergo an active but subclinical infection following exposure to infectious pancreatic necrosis (IPN) virus. Eight-week-old Atlantic salmon fry were fed live virus and the mortality and virus concentration in these fry were recorded over a period of 78 d following exposure. Yearling Atlantic salmon were inoculated intraperitoneally with IPN virus and the concentration of virus in the pancreas–intestine, kidneys, spleen, liver, and gonads of inoculated fish was determined over a period of 74 d following inoculation. Virus first appeared in the fry 3 d postinoculation (DPI) and virus titers reached a peak by 8 DPI. Low levels of virus persisted in fry sampled 78 DPI. Virus first appeared in the pancreas–intestine of Atlantic salmon 12 h postinoculation (HPI) and was detected in the kidneys, liver, and spleen by 72 HPI. Peak virus titers were reached in the pancreas–intestine by 72 HPI. Both the pancreas–intestine and kidneys continued to support viral growth frequently and appeared to be important reservoirs of virus. At no time was virus isolated from the gonads of inoculated yearling. Histological examination of yearlings revealed degenerative changes in the pancreatic acinar tissue and in some cases focal necrosis in the liver. No histological abnormalities were found in IPN-infected fry.

Key words: infectious pancreatic necrosis, Atlantic salmon, virus, histology

SWANSON, R. N., AND J. H. GILLESPIE. 1979. Pathogenesis of infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 36: 587–591.

Nous constatons que des alevins et des saumoneaux atlantiques (*Salmo salar*) de 1 an subissent une infection active mais subclinique après exposition au virus de la nécrose pancréatique infectieuse (NPI). On a donné à manger des virus vivants à des alevins de saumon atlantique âgés de 8 sem et enregistré sur une période de 78 d après exposition la mortalité et la concentration du virus chez ces alevins. Nous avons transmis par injection intrapéritonéale le virus de la NPI chez des saumoneaux de 1 an et mesuré la concentration du virus dans le pancréas–intestin, les reins, la rate, le foie et les gonades des poissons inoculés, sur une période de 74 d après inoculation. Le virus apparaît pour la première fois chez les alevins 3 d après inoculation (DPI) et les titres du virus atteignent un sommet à 8 DPI. Il y a encore des bas niveaux de virus dans des alevins échantillonnés 78 DPI. Le virus apparaît d'abord dans le pancréas–intestin des saumons atlantiques 12 h après inoculation (HPI) et est détecté dans les reins, le foie et la rate en dedans de 72 HPI. Les titres maxima du virus sont atteints dans le pancréas–intestin à 72 HPI. Le pancréas–intestin et les reins continuent à soutenir la croissance du virus et semblent être d'importants réservoirs pour le virus. Nous n'avons en aucun temps isolé le virus des gonades des saumoneaux de 1 an inoculés. L'examen histologique des poissons de 1 an révèle des changements dégénératifs dans le tissu acinaire pancréatique et, dans certains cas, une nécrose focale dans le foie. Nous n'avons observé aucune anomalie histologique chez les alevins infectés par la NPI.

Received September 21, 1978
Accepted January 31, 1979

Reçu le 21 septembre 1978
Accepté le 31 janvier 1979

INFECTIOUS pancreatic necrosis (IPN) is an acute, highly contagious virus disease of salmonid fishes often causing high mortality and significant economic loss in salmonid hatcheries throughout the world. Mortalities due to IPN have been reported most commonly in rainbow trout (*Salmo gairdneri*) and brook trout (*Salvelinus fontinalis*) although the Salmonidae as a whole seems to be susceptible to infection with IPN virus (McKnight and Roberts 1976). Although earlier descriptions of the

Printed in Canada (J5382)
Imprimé au Canada (J5382)

disease included references to IPN-associated mortalities in Atlantic salmon (*Salmo salar*) (Snieszko and Wolf 1958) and IPN virus has been isolated from clinically normal Atlantic salmon (MacKelvie and Artsob 1969), there have been no reports detailing the distribution of virus within infected Atlantic salmon.

The present study was undertaken to help elucidate the pathogenesis of IPN in Atlantic salmon. We believe continued study of the pathogenesis of this disease in terms of virus growth and distribution will ultimately lead to the development of more efficient techniques for IPN diagnosis.

Materials and methods — Monolayer cultures of RTG-2 (rainbow trout gonad) cells developed by Wolf and Quimby (1962) were used for virus titrations and stock virus preparation. The cultures were grown in Eagle's Minimum Essential Medium (Hank's balanced salt solution) supplemented with 12% fetal calf serum containing 100 IU penicillin, 100 μg streptomycin, and 25 IU fungizone/mL. The RTG-2 cells were purchased from the American Type Culture Collection and had been subcultured 50–60 times.

IPN virus was the American Type Culture Collection strain VR299 passaged 12 times in RTG-2 cells. Stock virus was prepared by serial passage of terminal dilutions and normally yielded 10^{-6}–10^{-7}TCID50 (50% tissue culture infective doses)/0.2 mL. RTG-2 cultures grown in 75-cm^2 Falcon flasks (Falcon Plastics, Oxnard, Calif.) were inoculated with 0.2 mL virus dilution and incubated at 20°C until advanced CPE occurred, usually 48–72 h later. At this time the cell cultures were subjected to three cycles of freezing and thawing and the virus-containing tissue culture fluids clarified by centrifugation at 2000 g for 15 min. Stock virus preparations were routinely passed through 0.45-μm membrane filters before storage at −70°C.

Viral infectivity of stock preparations and tissue homogenates was determined using microtiter technique and RTG-2 cells according to the method of Piper et al. (1973) and virus titers were expressed as TCID50/0.2 mL using the method of Karber (1931). The presence of IPN virus in tissue homogenates was confirmed by serum neutralization tests using high titer antiserum prepared in rabbits and the procedure reported by Wolf and Quimby (1969).

Fish infectivity tests — The Atlantic salmon used in this study were from certified IPN-free stock and were kindly supplied by the Tunison Laboratory of Fish Nutrition, Cortland, N.Y. Two hundred 8-wk-old Atlantic salmon fry (2–3 cm) were placed in a 209-L Living Stream Unit (Frigid Units, Toledo, Ohio) filled with spring water also obtained from Tunison Laboratory. The major chemical characteristics of this water were as follows: water hardness of 186–194 mg/L as $CaCO_3$, dissolved oxygen of 9.1 mg/L, pH of 7.9, and nitrates of 5.32–11.07 mg/L. The fry were maintained in a water temperature of 11–15°C and fed twice daily with commercial ration supplied by Tunison Laboratory. Feed was withheld for 24 h and the fry were fed a mixture of ration and cloned stock virus containing 10^{-7}TCID50/g ration. The ration–virus mixture was given to the fry twice over a period of 1 d resulting in a concentration of ~100TCID50/mL water in the tank. Groups of 5–10 fry were removed daily and processed for virus isolation and histopathology. Daily samples were terminated at 12 DPI with remaining samples taken at 14, 16, 18, 20, 39, and 78 DPI. Appropriate controls were carried out at the Tunison Laboratories under similar conditions.

Fifty yearling Atlantic salmon (10–15 cm) were distributed in 209-L and 302-L Living Stream Units containing spring water identical to that previously described. The salmon were maintained in a water temperature of 7–9°C and were fed once weekly with commercial ration. The fish were anesthetized with MS-222® (Kent Laboratories, British Columbia) at a dosage of 50 mg/L water and each given a 0.25-mL intraperitoneal injection of stock virus containing a total of 2.5×10^{-6}TCID50 IPN virus. Two control fish were inoculated with 0.25 mL tissue culture medium using identical techniques. Groups of three fish were removed on ½ (12 HPI), 1, 2, 3, and 4 DPI and a group of two fish was removed on 5 DPI. Remaining samples were removed on 6, 7, 8, 9, 10, 11, 12, 38, 57, and 74 DPI.

Virus isolations and histology — Virus isolations were obtained from salmon killed by overexposure to MS-222®. Whole fry were immediately placed in 10 vol of phosphate buffer solution (PBS), homogenized in glass tissue grinders, and stored in sealed vials at −70°C. Yearling salmon were dissected and organs aseptically removed included the kidneys (anterior and posterior), spleen, liver, gonads, and a segment of the ascending intestine including pyloric ceca and the pancreatic tissue, which will be referred to as pancreas–intestine. Each organ was removed with a sterile set of dissecting instruments to eliminate the possibility of cross contamination. Tissue samples were immediately placed in 10 vol of PBS, homogenized in glass tissue grinders, and stored in sealed vials at −70°C. Before titration in cell culture all tissue homogenates were centrifuged at 3000 *g* for 15 min to remove tissue debris and the virus-containing supernatant was treated with antibiotics as described by Amend and Pietsch (1972). Previous titrations indicated that no loss of virus titer occurred using this method. Tenfold dilutions of antibiotic-treated tissue homogenates were prepared in maintenance medium and titrated in microtiter plates according to the methods previously described.

Whole fry and small pieces of liver, spleen, kidneys, pyloric ceca, and intestinal tissue from IPN virus-inoculated and control salmon were placed in Bouin's fixative for 24 h, rinsed, and stored in 70% ethanol. Tissue specimens were embedded in paraffin, sectioned 7 μm, and stained with hematoxylin and eosin. Fry were decalcified, paraffin-embedded, sectioned longitudinally 7 μm, and stained with hematoxylin and eosin.

Results — Virus isolations — Atlantic salmon fry fed live IPN virus showed no clinical signs of IPN and the 17% cumulative mortality did not vary significantly from controls. Virus was isolated 3 DPI and reached a maximum titer of 10^{-5}TCID50/0.2 mL by 8 DPI (Fig. 1). No virus was isolated from fry sampled 11, 12, and 16 DPI, but a titer of $10^{-1.13}$TCID50/0.2 mL was isolated from fry sampled 78 DPI. Virus titers obtained from fry found dead did not vary significantly from those obtained from normal-appearing fry sampled at the same time.

Atlantic salmon yearling inoculated intraperitoneally with IPN virus failed to show evidence of disease and suffered no mortalities. Virus first appeared in the

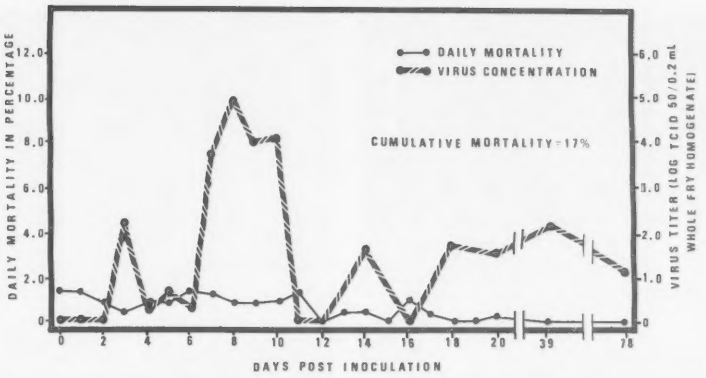

DAILY MORTALITY
VIRUS CONCENTRATION
CUMULATIVE MORTALITY = 17%
DAILY MORTALITY IN PERCENTAGE
VIRUS TITER (LOG TCID 50/0.2 mL WHOLE FRY HOMOGENATE)
DAYS POST INOCULATION

FIG. 1. Percent mortality and average virus concentration in 8-wk-old Atlantic salmon (*Salmo salar*) fry inoculated orally with 10^{-7}TCID50 IPN virus/g feed.

pancreas–intestine and was detected soon after in the kidneys, liver, and spleen (Fig. 2). A titer of $10^{-2.6}$TCID50/0.2 mL was present in the pancreas-intestine 12 HPI and this increased to $10^{-4.3}$TCID50/0.2 mL by 2 DPI. Virus titers up to $10^{-4.0}$TCID50/0.2 mL continued to be present in the pancreas–intestine. Virus was first detected in the kidneys on 2 DPI and a titer of $10^{-5.1}$TCID50/0.2 mL was reached by 4 DPI. No virus was detected in kidneys sampled 5 DPI, but virus titers up to $10^{-3.8}$TCID50/0.2 mL continued to be present after this. Virus was first detected in the spleen on 2 DPI and a titer of $10^{-5.2}$TCID50/0.2 mL was reached by 4 DPI. No virus was detected in spleens sampled 6-57 DPI, but a titer of $10^{-2.2}$TCID50/0.2 mL was present on 74 DPI. Virus was first detected in the liver on 3 DPI and a titer of $10^{-3.3}$TCID50/0.2 mL was reached by 4 DPI. Titers of $10^{-2.0}$ and $10^{-3.0}$TCID50/0.2 mL were present in livers on 10 and 57 DPI, respectively. Virus was not isolated from the gonads of infected salmon. All virus isolations were identified as IPN virus by the serum neutralization test.

Histopathology — Examination of longitudinally sectioned fry revealed no major histological changes occurring after exposure to IPN virus. However, microscopic changes in the pancreas of infected yearlings were seen in fish sampled 3-9 DPI. Focal areas of mononuclear infiltration were frequently observed in infected salmon and often this was seen in association with degeneration of pancreatic acinar cells. Acinar cell degeneration was generally confined to small areas and was characterized by loss of zymogen granules, pyknosis,

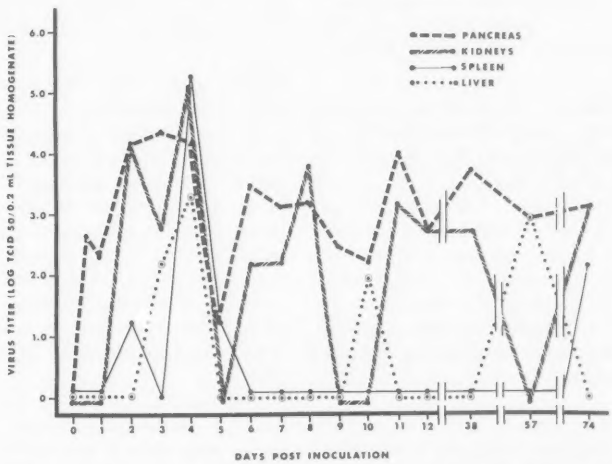


FIG. 2. Virus concentration in various organs of Atlantic salmon (*S. salar*) yearling inoculated intraperitoneally with 2.5×10^{-6}TCID50 IPN virus.

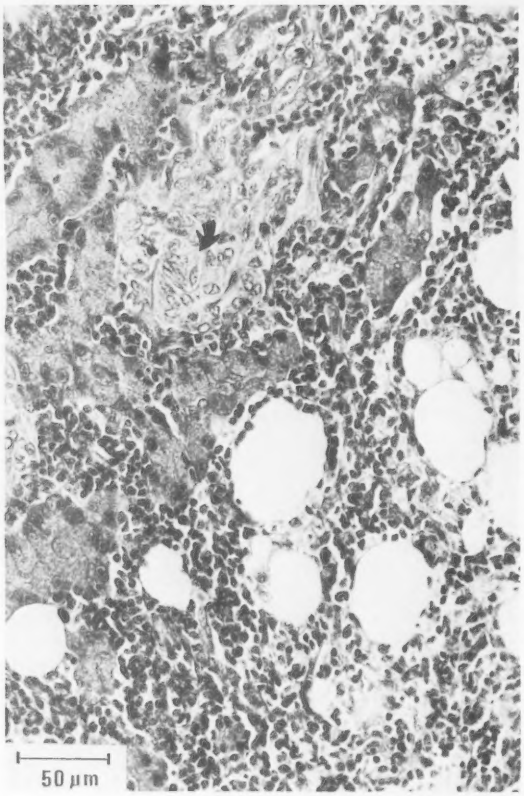


FIG. 3. Section of pancreas from yearling Atlantic salmon 9 d following intraperitoneal inoculation with IPN virus. Acinar cells are present in various stages of degeneration and a marked infiltration of mononuclear cells can be seen. Most of the inflammatory cells appear to be lymphocytes. Note the relative uninvolvement of islet tissues (arrow).

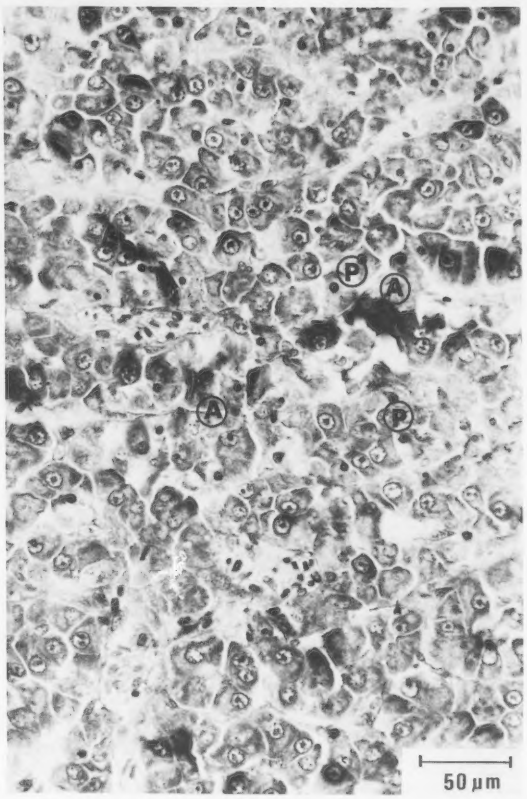


FIG. 4. Section of liver from yearling Atlantic salmon 3 d following intraperitoneal inoculation with IPN virus. Pyknotic nuclei (P) and cytoplasmic acidophilia (A) are obvious in degenerating hepatocytes. Several such areas of focal degeneration were found in this liver.

and karyorrhexis. Occasionally more severe pancreatic lesions were seen and these were characterized by extensive degeneration of acinar cells and marked infiltration of mononuclear cells (Fig. 3).

Histological changes were also evident in the livers of yearling sampled 3-4 DPI. These lesions consisted of focal areas of parenchymal cell degeneration characterized by acidophilia, pykonosis, and karyolysis of hepatocytes (Fig. 4). No histological changes were detected in kidney, spleen, or intestinal samples examined. Histological abnormalities were also absent in all tissues of control salmon examined.

Discussion — In the present study, IPN virus was isolated consistently from Atlantic salmon fry and yearling up to 78 and 74 DPI, respectively. Although limited virus replication may have occurred in the salmon, it did not result in clinical disease. MacKelvie and Artsob (1969) in one of the few reports of IPN virus isolation from Atlantic salmon, found a low incidence of isolation among salmon fry reared in Canadian hatcheries harboring IPN. None of the fry appeared to be diseased and the relatively small number of samples found to be virus positive was attributed to a low incidence of infection or the presence of virus in quantities too small to be detected. The present data suggest that IPN virus does replicate in Atlantic salmon tissue and raise the question of whether Atlantic salmon exposed to IPN virus at an earlier age or under more stressful conditions may be susceptible to clinical disease and mortality.

The absence of clinical disease in Atlantic salmon yearling following inoculation with IPN virus is consistent with what is known of the age susceptibility of other salmonid species to IPN. Mortalities due to IPN are greatly reduced in trout reaching the late fry and fingerling stage (Wolf 1972) and adult rainbow and

brook trout inoculated with IPN virus intraperitoneally suffer only a transient subclinical infection (Wolf and Quimby 1969). It is interesting to note that in the present study a generalized spread of virus in inoculated Atlantic salmon began about 2 DPI and was followed by increased titers in all organs examined with exception of the gonad. This may well have been a critical period in the infection; had virus growth not been controlled by the salmon at this point it is likely that clinical disease would have resulted. Mortalities following intraperitoneal inoculation of IPN virus in rainbow trout fry begin ~3 DPI (Lightner 1971) and stress-induced losses in older fish also begin to occur 3 d after the initial stress (Roberts and McKnight 1976). This corresponds exactly to the period of increased titers and generalized virus spread seen in the Atlantic salmon yearlings. The mechanisms involved in the eventual control of this infection are unknown, as are the means of viral persistence.

From the histopathological findings of this study, it appears that IPN infection in yearling Atlantic salmon can result in varying degrees of pancreatic degeneration. In most cases the pancreatic degeneration was mild and it is doubtful that normal pancreatic function was impaired to any great extent. The occurrence of a more severe pancreatic lesion, however, demonstrates that IPN virus infection in the Atlantic salmon can result in serious histopathological changes. The importance of the liver lesions observed is more questionable. Hepatic necrosis has been reported in IPN-infected trout fry (Lightner 1971; Sano 1971) but has not been a consistent finding nor is it considered to be a lesion specific for IPN. More work is needed before the significance of the liver changes can be properly ascertained.

The absence of detectable lesions in the Atlantic salmon fry was surprising. Whole fry homogenates yielded virus titers comparable to those found in yearling tissues and the susceptibility of other salmonids to IPN is known to decrease with age (Wolf 1972). The fact that intraperitoneally inoculated yearlings exhibited pathological changes while orally inoculated fry did not may have been due to the difference in inoculation methods employed. Intraperitoneal inoculation of IPN virus in rainbow trout fry has been reported to yield a higher incidence of disease and more consistent results than oral inoculation (Lightner 1971).

Acknowledgments — This research was supported by Grant No. 04-7-158-44009, New York Sea Grant Institute, SUNY/Cornell University.

AMEND, D. F., AND J. P. PIETSCH. 1972. An improved method for isolating viruses from asymptomatic carrier fish. Trans. Am. Fish. Soc. 101: 267–269.

KARBER, G. 1931. Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Arch. Exp. Pathol. Pharmakol. 162: 480–483.

LIGHTNER, D. 1971. Infectious pancreatic necrosis (IPN): Its pathogenesis and morphology of the virus in rainbow trout. Ph.D. Dissertation, Colorado State University, Denver, Colo. (Microfilms, Ann Arbor, Mich.)

MACKELVIE, R. M., AND H. ARTSOB. 1969. Infectious pancreatic necrosis virus in young salmonids of the Canadian Maritime Provinces. J. Fish. Res. Board Can. 26: 3259–3262.

MCKNIGHT, I. J., AND R. J. ROBERTS. 1976. The pathology of infectious pancreatic necrosis. I. The sequential histopathology of the naturally occurring condition. Br. Vet. Jour. 132: 76–85.

PIPER, D., B. L. NICHOLSON, AND J. DUNN. 1973. Immunofluorescent study of the replication of infectious pancreatic necrosis virus in trout and Atlantic salmon cell cultures. Infect. Immun. 8: 249–254.

ROBERTS, R. J., AND I. J. MCKNIGHT. 1976. The pathology of infectious pancreatic necrosis. II. Stress-mediated recurrence. Br. Vet. Jour. 132: 209–214.

SANO, T. 1971. Studies on viral diseases of Japanese fishes. II. Infectious pancreatic necrosis of rainbow trout: pathogenicity of the isolants. Bull. Jpn. Soc. Sci. Fish. 37: 499–502.

SNIESZKO, S. F., AND K. WOLF. 1958. Infectious pancreatic necrosis of salmonid fishes (acute catarrhal enteritis). U.S. Dep. Inter., Fish. Wildl. Ser. Fish Leafl. 453.

WOLF, K. 1972. Advances in fish virology: A review 1966–1971. *In* L. E. Mawdesley-Thomas [ed.] Diseases of fish (Symposium Zool. Soc. Lond., No. 30). Academic Press, London and New York.

WOLF, K., AND M. C. QUIMBY. 1962. Established eurythermic line of fish cells in vitro. Science 135: 1065–1066.

1969. Infectious pancreatic necrosis: Clinical and immune response of adult trouts to inoculation with live virus. J. Fish. Res. Board Can. 26: 2511–2516.

Rapid Decompression Stress Accelerates Fibrinolysis in Fingerling Salmon[1]

JOHN J. WOODWARD, EDMUNDO CASILLAS, LYNWOOD S. SMITH

Fisheries Research Institute, University of Washington, Seattle, WA 98195, USA

AND BRIAN G. D'AOUST

Virginia Mason Hyperbaric Center, Seattle, WA 98101, USA

WOODWARD, J. J., E. CASILLAS, L. S. SMITH, AND B. G. D'AOUST. 1979. Rapid decompression stress accelerates fibrinolysis in fingerling salmon. J. Fish. Res. Board Can. 36: 592–594.

Rapid decompression of fingerling coho salmon (*Oncorhynchus kisutch*) after simulated dives of 30.5- and 40.5-msw (metres seawater) increased the activity of the fibrinolytic system. Anticoagulated blood samples were analyzed for fibrinolytic activity by measuring the rate of lysis after clotting the blood with a thrombin solution. Immediately and at 1 h postdecompression from 30.5 msw the lysing rate was above normal but returned to normal within 3 h and remained stable for ~24 h, but at 48 h it was depressed. The activity of the fibrinolytic system appeared to follow that of the coagulation system, indicating that the lytic system of fishes is sensitive and responsive to stress-induced changes in hemostatic parameters.

Key words: Oncorhynchus kisutch, fibrinolysis, decompression stress, bubbles, coagulation

WOODWARD, J. J., E. CASILLAS, L. S. SMITH, AND B. G. D'AOUST. 1979. Rapid decompression stress accelerates fibrinolysis in fingerling salmon. J. Fish. Res. Board Can. 36: 592–594.

La rapide décompression de fingerlings de saumon coho (*Oncorhynchus kisutch*) après plongées simulées à des profondeurs de 30,5 et 40,5 msw (mètres d'eau de mer) résulte en une augmentation de l'activité du système fibrinolytique. L'activité fibrinolytique a été déterminée dans des échantillons de sang contenant un anticoagulant en mesurant la vitesse de lyse après coagulation du sang à l'aide d'une solution de thrombine. Immédiatement et 1 h après décompression de 30,5 msw, la vitesse de lyse est plus grande que normale mais retourne à la normale en dedans de 3 h, demeure stable durant ~24 h. Après 48 h toutefois, elle avait diminué. L'activité du système fibrinolytique semble suivre celle du système de coagulation, ce qui indique que le système lytique des poissons est sensible et répond à des changements dans les paramètres hémostatiques provoqués par le stress.

Received September 20, 1978
Accepted February 7, 1979

Reçu le 20 septembre 1978
Accepté le 7 février 1979

EFFECTS of a decompression stress on the coagulation system of fish have been studied by Casillas et al. (1975, 1976) and Casillas (1978). Little attention, however, has been given to the effects of stress on the fibrinolytic system of fish. The lytic system, an essential part of the hemostatic complex in mammals, dissolves clots to prevent prolonged stasis in injured areas where bleeding has been controlled and repair has begun (Harker 1974). Although the fibrinolytic system in mammals has been characterized in great detail, assessment of lytic activity and adaptation of current methodology to studies involving fish are limited.

The need to study the fibrinolytic system arose with the discovery by Casillas et al. (1975) that consumptive coagulopathy might be occurring following rapid decompression from deep depths — 30.5–40.5 msw (metres seawater). Wolf (1973) included increased fibrinolytic activity as one of the indices used to confirm consumptive coagulopathy.

Previous studies by Doolittle and Surgenor (1962) and Murtaugh et al. (1973) indicated the existence of a powerful lytic agent in the blood of fish, but these presented very little quantitative information. Hougie (1971) reported no lysis activity in spawning Pacific salmon. The present experiment was designed to develop a rapid method to assess fibrinolysis in fish and to quantify its activity with respect to stress, in particular with gas-saturated decompression stress.

Materials and methods — Fingerling (100–150 mm fork length) coho salmon (*Oncorhynchus kisutch*) were maintained in water troughs with a single-pass system using Lake Washington water. Fish were kept at least 1 mo before testing and were fed every other day with Oregon Moist Pellets (Moore-Clark Company, La Conner, Wash.). Feeding was terminated 24 h before any test. Water temperatures ranged from 12 to 15°C.

[1]Contribution No. 499, College of Fisheries, University of Washington.

Printed in Canada (J5378)
Imprimé au Canada (J5378)

Fish were subjected to air saturation dives (Beyer et al. 1976) simulating depths of 30.5 and 40.5 msw using a hyperbaric chamber (D'Aoust and Seibold 1976) with a static 8-L water volume and flowthrough gas under pressure. Approximately eight fish were compressed to a predetermined depth at a rate of 30.5 msw/min and maintained at depth for 90 min. Fish were then decompressed at a rate of 30.5 msw/min to ambient pressure and placed in fresh lake water until time for blood sampling.

Blood samples were taken immediately after decompression, and 1, 3, 24, and 48 h later. For sampling, fish were individually placed in a 100 mg/L unbuffered anesthetic solution of MS-222®. Blood was obtained by severing the caudal peduncle and collecting the blood in plastic 1-mL syringes containing a 3.8% sodium citrate solution (9:1, blood:citrate). By means of a siliconized capillary pipette (1% siliclad, Clay Adams), 0.5 mL whole blood was immediately removed from the syringe and placed into a siliconized 6 × 50-mm test tube containing 20 μL of a 40 NIH units/mL solution of topical bovine thrombin (Parke-Davis). The blood clotted immediately, retracted from the walls of the tubes, and began lysing from the bottom. Progress of lysis was observed at 10-min intervals. When no change was seen in the length of clot during a 10-min period, lysis was defined as complete. The height of blood lysed and the time required for it were then recorded and used as the basis for calculating fibrinolytic rate, in terms of millimetres blood lysed per minute. Maximum time for observing clot lysis was set arbitrarily at 240 min because most samples lysed completely within this time.

All statistical comparisons were made using a one-tailed Mann–Whitney U-test at a significance level of 0.05 (Zar 1974).

Results — Table 1 shows the effect of decompression on fibrinolytic activity. Lysing rates for the normal and chamber control fish were not statistically different. At 1 h postdecompression from 30.5 msw, the lysing rate was statistically greater than normal. Lysing rates for the 30.5-msw decompression returned to normal 3-h postdive and remained there until 48 h when a statistically significant depression occurred. The 40.5-msw decompression immediately increased the lysing rate to a statistically different level from normal. Data from the 40.5-msw decompression were incomplete because the fish died within 10 min after decompression.

TABLE 1. Lysing rates (mm blood lysed/min) of fingerling coho salmon (*Oncorhynchus kisutch*) following rapid decompression. Tests compared to normal with one-tailed Mann–Whitney U-test at 0.05 significance level.

	+1 min	+1 h	+3 h	+24 h	+48 h
Normal	0.07	—	—	—	—
	0.09				
	0.13				
	0.17				
	0.18				
	0.20				
	0.25				
	0.29				
Chamber controls	0.07	0.10	—	0.13	0.09
	0.12	0.11		0.17	0.10
	0.16	0.13		0.17	0.13
	0.17	0.14		0.21	0.17
	0.20	0.14		0.28	0.17
	0.21	0.15		0.28	0.17
		0.16			0.20
		0.17			0.28
30.5 msw	0.13	0.20[b]	0.10	0.12	0.04[c]
	0.14	0.21	0.15	0.13	0.05
	0.25	0.23	0.15	0.13	0.07
	0.27	0.23	0.17	0.16	0.07
	0.29	0.29	0.20	0.16	0.07
	0.40	0.32	0.20	0.17	0.07
	0.45	0.45	0.21	0.24	0.07
				0.25	0.07
					0.11
					0.14
					0.16
					0.17
					0.18
40.5 msw	0.19[a]	[d]	[d]	[d]	[d]
	0.23				
	0.23				
	0.25				
	0.30				
	0.36				

[a]Lysing rates greater than normal, $0.025 < P(U) < 0.05$.
[b]Lysing rates greater than normal, $0.01 < P(U) < 0.025$.
[c]Lysing rates less than normal, $0.005 < P(U) < 0.01$.
[d]No survivors.

Discussion — Decompression from a gas saturation dive increased the lysing rate of whole blood in vitro. Although only two depths were investigated, the lysing rate is probably depth-related because the coagulation system in fish was shown to be enhanced by greater decompression depths (Casillas 1978).

Lysing rates showed increases after the 30.5-msw decompression but required 1 h before becoming statistically greater than normal values, showing individual responses to the stress. At 40.5 msw, all fish showed an immediate significant increase in the lysing rate, indicating a more severe stress which overcame individual variations (Beyer et al. 1976).

Recovery to normal lysing rates at 3 h postdive and the subsequent depression to subnormal values at 48 h postdive for the 30.5-msw decompression can be viewed as an overshoot by the hemostatic system in an attempt to restore a normal level of fibrinolytic activity. Casillas (1978) reports a similar oscillating pattern in fingerling coho salmon prothrombin times in response to decompression from 30.5 and 40.5 msw. Any change in the coagulation activity would be correlated with change in the fibrinolytic activity, as the two systems are likely interrelated (Harker 1974).

Increased activity of the fish's fibrinolytic system follows that of the coagulation system. Casillas (1978) showed that rainbow trout (*Salmo gairdneri*) respond to the compression phase of a gas saturation dive by releasing epinephrine and norepinephrine. If fish re-

spond to catecholamines as mammals do (Ingram et al. 1977; Hawkey et al. 1975), this release likely caused the noted increases of circulating thrombocytes and fibrinogen and a shortening of prothrombin times, thereby preparing the coagulation system for any vascular injury (Casillas 1978).

If consistent in fish, decompression with subsequent bubble formation and resulting endothelial damage probably precipitates thrombocyte aggregation and plasminogen-coated fibrin deposition. Conversion of plasminogen to plasmin by one or more substances — thrombin, for instance — might initiate lysis of the clot (Harker 1974). The increased lysing rate for decompressed fish could indicate increased circulating plasminogen levels, and therefore more potential plasmin for lysis. Few data, however, are available concerning these fibrinolytic factors in fish.

Although fingerlings were used in this study, similar responses of the lytic system after decompression would be expected in nonspawning adults, as the adult fish's coagulation system and that of the fingerling fish respond similarly to stress (Casillas et al. 1975; Casillas 1978).

The results of this study are the first clear indication that the fibrinolytic system of fish is responsive to stress-induced changes in the coagulation system and is an important part of the hemostatic complex. Many types of stressors, including exercise, disease, shock, and surgery, are known to evoke changes in the clotting systems of mammals (Ferguson and Guest 1974; Iatridis and Ferguson 1963; Ygge 1970; Rizza and Eipe 1971), and Casillas and Smith (1977) showed that the coagulation system of rainbow trout was enhanced by a simulated hooking stress. The lysing rate method described in this paper might provide a rapid, simple technique to determine the severity of a stressor imposed on fish in which perturbations of the blood coagulation system are expected.

Acknowledgments — This research was supported by National Institutes of Health Grant No. HL-16254.

BEYER, D. L., B. G. D'AOUST, AND L. S. SMITH. 1976. Decompression-induced bubble formation in salmonids: Comparison to gas bubble disease. Undersea Biomed. Res. 3: 321–338.

CASILLAS, E. 1978. Hematological response of fish to independent changes in hydrostatic and gas pressure. Ph.D. Thesis, Univ. Washington, Seattle, Wash.

CASILLAS, E., S. E. MILLER, L. S. SMITH, AND B. G. D'AOUST. 1975. Changes in hemostatic parameters in fish following rapid decompression. Undersea Biomed. Res. 2: 267–276.

CASILLAS, E., AND L. S. SMITH. 1977. Effect of stress on blood coagulation and haematology in rainbow trout (*Salmo gairdneri*). J. Fish. Biol. 10: 481–491.

CASILLAS, E., L. S. SMITH, AND B. G. D'AOUST. 1976. The response of fish blood cells, particularly thrombocytes, to decompression. Undersea Biomed. Res. 3: 273–281.

D'AOUST, B. G., AND H. SEIBOLD. 1976. An adjustable two-compartment pressure chamber for study of aquatic organisms. Undersea Medical Society, North Pacific Chapter, Proc. 3rd Annu. Meet., p. 6–8.

DOOLITTLE, R. F., AND D. M. SURGENOR. 1962. Blood coagulation in fish. Am. J. Physiol. 203: 964–970.

FERGUSON, E. W., AND M. N. GUEST. 1974. Exercise, physical conditioning, blood coagulation and fibrinolysis. Thromb. Diath. Haemorrh. 31: 63–71.

HARKER, L. A. 1974. Hemostasis manual, 2nd ed. F. A. Davis Co., Philadelphia, PA.

HAWKEY, C. M., B. J. BRITTON, W. G. WOOD, M. PEELE, AND M. H. IRVING. 1975. Changes in blood catecholamine levels and blood coagulation and fibrinolytic activity in response to graded exercise in man. Br. J. Haematol. 29: 337–384.

HOUGIE, C. 1971. Coagulation changes in healthy and sick Pacific salmon. Adv. Exp. Med. Biol. 22: 89–102.

IATRIDIS, S. G., AND J. H. FERGUSON. 1963. Effect of physical exercise on blood clotting and fibrinolysis. J. Appl. Physiol. 18: 336–344.

INGRAM, G. I. C., R. V. JONES, E. J. HERSHGOLD, W. E. DENSON, AND J. R. PERKINS. 1977. Factor-VIII activity and antigen, platelet count, and biochemical changes after adrenoreceptor stimulation. Br. J. Haematol. 35: 81–100.

MURTAUGH, P. A., J. E. HALVER, AND J. A. GLADNER. 1973. Cross-linking of salmon fibrinogen and fibrin by factor XIII and transglutaminase. Biochem. Biophys. Res. Commun. 54: 849–855.

RIZZA, C. R., AND J. EIPE. 1971. Exercise, factor VIII, and the spleen. Br. J. Haematol. 20: 629–635.

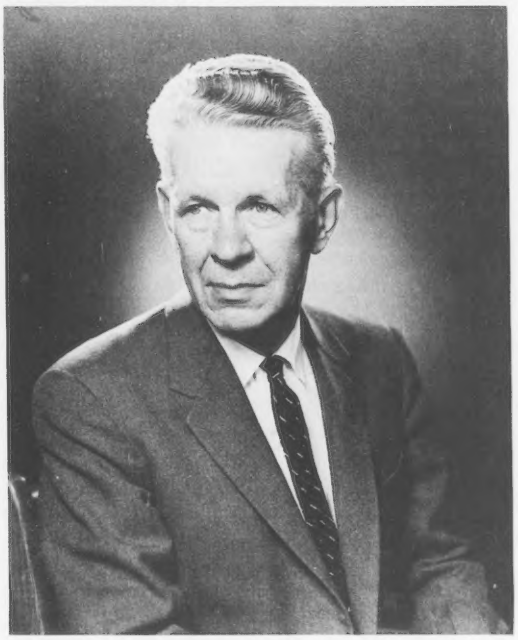
WOLF, P. L. 1973. Disseminated intravascular coagulation: Principles of diagnosis and management, p. 17–44. *In* G. Schmer and P. E. Strandjord [ed.] Coagulation: Current research and clinical applications. Academic Press, New York, N.Y.

YGGE, J. 1970. Changes in blood coagulation and fibrinolysis during the post-operative period. Am. J. Surg. 119: 225–232.

ZAR, J. H. 1974. Biostatistical analysis. Prentice-Hall, New Jersey, p. 108–112.

Neal Marshall Carter

1902-1978



Neal Carter was born in Vancouver, British Columbia, on December 14, 1902. He attended the University of British Columbia, where he received the degrees of B.A.Sc. in 1925 and M.A.Sc. in 1926. Further study at McGill University resulted in a Ph.D. degree in 1928, after which an NRC Travelling Fellowship enabled him to spend a year at the Kaiser Wilhelm Institut in Dresden.

About this time the Director of the Pacific Biological Station, Dr W. A. Clemens, was casting about for a staff member to undertake fulltime work in oceanography. Such studies had been pursued seasonally on the Strait of Georgia for half a decade by volunteer investigators from the University of British Columbia. However, it was time to go farther afield, and particularly to have year-round observations. On hearing about Carter, Dr Clemens wrote to him in Germany offering him the position and suggesting that if interested, he might visit the Board's Halifax and St. Andrews Stations on his way back from abroad. Although almost "broke" after a year on the modest NRC stipend, Neal managed to inspect the work of the eastern stations before reaching Nanaimo in September of 1930.

At that time Hecate Strait, the mainland inlets, and the open ocean off the British Columbia coast were all practically unknown oceanographically. The Station had no boat suitable for extended work in exposed waters, so Dr Carter selected the inlets for intensive study. These long Norwegian-style fjords are avenues down which many millions of young salmon make their way to sea each year, as well as supporting resident stocks of fishes and prawns. The methodology of chemical oceanography was still in a primitive state, and much time had to be spent in adapting newly discovered procedures and standardizing old ones. In addition to oceanography, while at Nanaimo Neal carried out analyses of fresh and canned fish products and studied the technique of salting herring.

In 1933 Neal's oceanographic career was interrupted when he was appointed Director of the Pacific Fisheries Experimental Station at Prince Rupert. In 1942 this Station's premises were requisitioned for military use, and staff and equipment were moved to Vancouver. As Director, Neal felt that his primary responsibilities were to provide a good climate for research by the staff, to maintain close liason with the fishing industry, and to make research results available promptly and in usable form. The "Progress Reports" of the Pacific stations of the Biological Board had been started in 1929, but soon after reaching Prince Rupert Neal became their editor and continued in this position through 1962. In 1935 he initiated the "Industrial Memoranda" of the Technological Station, later called Circulars. But in addition he found time for work on such problems as testing fishing net preservatives, concentrating clam nectar by freezing, making pearl essence from herring scales, determining the scorch temperature in drying fish meals, preparation of brines and dips for freezing, herring resource use problems, and preservation of salmon eggs for bait. He even wrote an article on biblical references to fish and fishery products.

From June to December of 1946 Neal was on special assignment in Japan, then under military occupation. While there he assessed wartime developments in Japanese fisheries and provided technical assistance in getting their industry back on its feet during the difficult postwar period.

Painstaking and meticulous in whatever he did, Dr Carter made an ideal editor, and this became one of

Printed in Canada (J5501)
Imprimé au Canada (J5501)

his major duties when he went to Ottawa in 1955 as Special Assistant to Dr J. L. Kask, the Chairman of the Fisheries Research Board. In 1954 the amount of material printed in the Board's central publications had doubled, so Neal was appointed Associate Editor with responsibility for papers concerned with products research and oceanography, and for handling all of the direct liason with the printers. In addition, of course, he had a hand in many of the multitudinous chores and decisions that are required of public servants sitting close to the seat of power.

In 1962 Dr Carter retired and moved back to the west coast, but this did not end his service to fishery research. As independent contractor, he for many years compiled the annual index to all Board publications — both in-house and those in "outside" journals and books. Even more important, he prepared two consolidated indexes, one for 1900–64 and one for 1965–72.

No biography of Neal Carter could be complete without mention of his principal avocation — mountaineering. He was a member of the B.C. Mountaineering Club from 1920 to 1926, and afterward of the Alpine Club of Canada. The latter Club made him an Honorary Member in 1974, in recognition of his numerous climbing achievements and also of the numerous executive and editorial jobs he had performed for it. Unlike many alpinists, Neal was not content simply to climb every peak in sight; rather, he took time to prepare maps and guides for the areas he frequented. He even became proficient in the difficult art of preparing contoured maps from ground photography, in the days before air photographs were available. Some of his maps of parts of British Columbia's Coast Range are still the best available. One result of these activities was his election as a Fellow of the Royal Geographic Society. Another was his appointment as Alpine Features Adviser to Canada's Permanent Committee of Geographic Names.

It was not mountaineering, however, that brought Dr Carter's life to an abrupt end on March 15, 1978; instead, his heart gave out while he was snorkelling in Barbados. Neal's first wife, Peggy, the companion of many of his mountain trips, predeceased him in 1973. He is survived by his second wife, Dorothea, sons David and Bruce, and daughter Louise.

W. E. RICKER

A Coastal Marine Ecosystem. Simulation and Analysis. *J. N. Kremer and S. W. Nixon. Springer-Verlag, New York, Heidelberg, Berlin. 1978. xi + 217 p. $32.00.*

The development of scientific understanding involves two fundamental components — experimentation and interpretation. Science begins with observation and the collection of information, but then, as the data suggest patterns indicative of underlying structure, an interpretive framework develops. In ecology, as in all other sciences, interpretation has come to constitute an ever larger fraction of the total scientific effort. This trend is reflected in a growing body of literature with a largely or even exclusively theoretical orientation. *A Coastal Marine Ecosystem* is a book of this genre and might be considered its acme — in over 200 pages you will find no new data, although the authors refer extensively to experimental work published elsewhere. This has the beneficial aspect of letting the empirical aspects of the study appear in the refereed literature where they can be evaluated separately by specialists in various component areas.

A book-length monograph like this one is becoming the fashionable way for theorists to present complex systems models to a general audience, as with Steele's North Sea model. Large-scale simulations are difficult to present adequately as journal articles, and it is generally the weak points that are obscured in the process of condensation. Of course, full disclosure brings with it risks — in a multidisciplinary subject, every specialist is bound to find some point of dissatisfaction.

The adequacy of a model depends on several factors, including the accuracy of the parameters, and of course the correctness of the conceptual framework. Most aspects of the Narragensett Bay model seem to be dealt with adequately, but there is room for question in several areas, including the treatment of stochastic effects and grazing rates. There is also some ambiguity about what the model is supposed to do, which makes it hard to judge how well it does it.

The only stochastic element in the model is cloud cover, which determines the amount of solar radiation reaching the surface. The way in which this is calculated can only be described as circuitous. Since a quite adequate set of insolation data was apparently available, and since the model uses 3-day running averages of radiation, the obvious approach is to fit a statistical model to 3-day averages of real data. Instead, the authors compute maximum insolation from an astronomical model, multiply by an arbitrary factor of 0.7 to obtain better agreement with the data, and use cloud cover (rather than actual radiation) data to obtain the statistical distribution of the fraction of radiation reaching the surface. The results, as shown by their figure 10 and discussed in the text, are in poor agreement with the empirical distribution. Furthermore, even if the radiation model gave a good fit on a day-to-day basis, no effort was made to consider autocorrelations in weather patterns from day to day, so it would not necessarily hold that the distribution of 3-day running averages in the model is realistic. There is also the question of whether inclusion of correlations between radiation and temperature, mixing, and runoff would have any significant consequences.

The treatment of grazing rates raises several disturbing issues. The purely mathematical material between Eqs. (18) and (21) consists merely of rewriting Ivlev's equation in different symbols and does not lead to "a general form." The authors conclude that Ivlev's equation does not fit the data, but instead of using a different theoretical equation or a purely empirical approach they adjust the Ivlev curve by multiplying one of the parameters, k, by 2 to obtain "a more satisfactory pattern"! They justify this incredible approach to parameter estimation by explaining that "statistical methods of fitting equations . . . to linearized data may weight the points with greater numerical value more heavily, thus biasing against the low food concentrations which are most interesting in evaluating filtering rates." (p. 66). True, but easily avoided by the use of weighted or nonlinear regression techniques. The existence of some inappropriate statistical methods does not justify the rejection of all statistics! Furthermore, the sensitivity analysis on p. 184–185 shows that this correction has a very large effect on the model output — according to figure 72, the doubling of k reduces the phytoplankton biomass by a factor of up to 3 and increases the zooplankton biomass by a factor sometimes more than 10. A further doubling of k seriously destabilizes the model, and it is hard to feel confident in the output of a model so sensitive to such a crudely estimated parameter.

These concerns might not be serious if the model were conceived of as a purely theoretical exercise, as suggested by the preface and Chapter 1. Consistent with this approach, the emphasis is on plankton-nutrient dynamics with only brief consideration of bacteria, fish, and other compartments. However, it is disturbing to come to Chapter 12 and see a discussion of the possible uses of the model for environmental management which ignores some of the caveats expressed in Chapter 10 and elsewhere. The model is clearly intended for further development, and it is evident that research is continuing in areas not well covered in the present version. Some of these areas are considered in the section on sensitivity analysis — for example, the effects of menhaden predation are dealt with by an ingenious functional sensitivity analysis in which a number of different hypothetical feeding behaviors is assumed.

From a mathematical point of view the work has little new to offer. I have already commented on the authors' simplistic approach to statistics and their predilection for making arbitrary adjustments rather than estimating parameters properly. The prospective programer would be better off relying on an introductory text on coding. For example, the use of EQUIVALENCE statements to pack small arrays inside a large one is recommended for efficient communication with subroutines. This will save some time, but the standard method of using COMMON blocks is even faster and avoids constraints on the order of subscripts.

The text is written in an unfortunate melange of mathematical and FORTRAN terminology. The symbols "·" and "*" are used interchangeably to denote multiplication, and "AMIN1" is used instead of "min" in a mathematical expression. It is also lamentable that in writing FORTRAN code the authors use non-ANSI notation and ignore type conventions (e.g. real IOPT, LUXSI, MXLIM,

NTOT, integer ELT). Editors should insist that mathematical texts be written in standard mathematical notation and that computer code be expressed in consistent and universal form — for FORTRAN that means ANSI only.

Although any reader is bound to have reservations about parts of this book, this does not detract from the overall value of the work. Kremer and Nixon have presented their model in explicit, and thus vulnerable, detail, but this is infinitely preferable to the traditional opaque summary accompanied by an undocumented listing of the full simulation program. The analysis section deserves special praise for the extensive discussion of system behavior, although the otherwise fine section on sensitivity analysis should have been put together with an assessment of confidence ranges for the parameter values. The book is certainly recommended for anyone working on a simulation modeling exercise, but it is likely to prove of even greater value to experimenters who would like a conception of how their research could be incorporated in a holistic ecological model.

WILLIAM SILVERT

Department of Fisheries and Oceans,
Marine Ecology Laboratory,
Bedford Institute,
P.O. Box 1006,
Dartmouth, N.S. B2Y 4A2

(R225)

COMING NEXT MONTH/À PARAÎTRE LE MOIS PROCHAIN

Karyotypic and evolutionary relationships of the Yellowstone (*Salmo clarki bouvieri*) and west-slope (*S. c. lewisi*) cutthroat trout **E. J. Loudenslager and G. H. Thorgaard**

Physiology of salinity-dependent naphthalene toxicity in *Fundulus heteroclitus* **W. M. Levitan and M. H. Taylor**

Physiological and biochemical aspects of ozone toxicity to rainbow trout (*Salmo gairdneri*) **G. A. Wedemeyer, N. C. Nelson, and W. T. Yasutake**

Acute ammonia toxicity and ammonia excretion in rainbow trout (*Salmo gairdneri*) **B. A. Hillaby and D. J. Randall**

Population dynamics of spiny dogfish (*Squalus acanthias*) in British Columbia waters **C. C. Wood, K. S. Ketchen, and R. J. Beamish**

Rate of loss of ammonia from water to the atmosphere **R. R. Weiler**

Phytoplankton ecology of the Strait of Georgia, British Columbia **J. G. Stockner, D. D. Cliff, and K. R. S. Shortreed**

Depression of pH in lakes and streams in central Ontario during snowmelt **D. S. Jeffries, C. M. Cox, and P. J. Dillon**

Plaque-forming cells and humoral antibody in rainbow trout (*Salmo gairdneri*) induced by immersion in a *Yersinia ruckeri* O-antigen preparation **D. P. Anderson, B. S. Roberson, and O. W. Dixon**

Perspectives

Bioeconomics of commercial fisheries management **C. L. Mitchell**

Notes

Role of tubificid worms on nitrogen transformations in stream sediment **L. Chatarpaul, J. B. Robinson, and N. K. Kaushik**

Decrease in mercury content of fishes in Ball Lake, Ontario, since imposition of controls on mercury discharges **F. A. J. Armstrong and D. P. Scott**

Intertidal catastrophes: effect of storms and hurricanes on intertidal benthos of the Minas Basin, Bay of Fundy **R. K. Yeo and M. J. Risk**

Composition of granules from kidneys of rainbow trout (*Salmo gairdneri*) with nephrocalcinosis **D. C. Gillespie and R. E. Evans**

Carbon isotope ratios of particulate organic matter in the Gulf of St. Lawrence **F. C. Tan and P. M. Strain**

Chronic exposure of trout to waters with naturally high selenium levels: effects on transfer RNA methylation **I. I. Kaiser, P. A. Young, and J. D. Johnson**

Density estimates of *Mysis relicta* in Lake Michigan **N. E. Grossnickle and M. D. Morgan**

RECENT PUBLICATIONS/RÉCENTES PUBLICATIONS

The number following each title in each series refers to the establishments listed at the end of the section. Inquiries concerning any particular item should be directed to the issuing establishment.

Les nombres qui suivent les titres de chacune des séries désignent les établissements suivants à la fin de la section. Pour toute demande de renseignements concernant un rapport particulier, il faut s'adresser au service responsable.

Technical Report Series

Technical Reports are research documents that are of sufficient importance to be preserved, but which for some reason are not appropriate for primary scientific publication. Inquiries concerning any particular Report should be directed to the issuing establishment.

790. ROSENBERG, D. M. 1978. Practical sampling of freshwater macrozoobenthos: a bibliography of useful texts, reviews, and recent papers. 15 p. (4)

791. OLIVER, D. R., D. MCCLYMONT, AND M. E. ROUSSEL. 1978. A key to some larvae of Chironomidae (Diptera) from the Mackenzie and Porcupine River watersheds. 73 p. (4)

Rapports techniques

Les rapports techniques sont des documents de recherche qui revêtent une assez grande importance pour être conservés mais qui, pour une raison ou pour une autre, ne conviennent pas à une publication scientifique de fonds. Pour toute demande de renseignements concernant un rapport particulier, il faut s'adresser au service responsable.

792. DRINNAN, R. E., AND W. B. STALLWORTHY. 1979. Oyster larval populations and assessment of spatfall, Bideford River, P.E.I., 1958. 32 p. (8)

793. DRINNAN, R. E., AND W. B. STALLWORTHY. 1979. Oyster larval populations and assessment of spatfall, Bideford River, P.E.I., 1959. 19 p. (8)

Manuscript Reports

This series includes unpublished preliminary reports and data records not intended for general distribution. They should not be referred to in publications without clearance from the issuing establishment and without clear indication of their manuscript status.

1440. MOSHENKO, R. W., AND D. V. GILLMAN. 1978. Creel census and biological investigation on lake trout, *Salvelinus namaycush* (Walbaum), from Great Bear and Great Slave Lake, Northwest Territories, 1975–76. 37 p. (4)

1441. PEPPAR, J. L. 1977. Angling survey, crown open water, Little Main Restigouche river, New Brunswick. 24 p. (8)

1442. HOURSTON, A. S., AND R. D. HUMPHREYS. 1978. Preliminary forecast of the available roe catch from British Columbia herring management units in 1978 and assessments of abundance in 1977. 11 p. (1)

1443. SIMPSON, K. S., J. R. CARMICHAEL, AND C. GROOT. 1978. Index to the Pacific Biological station archives. 95 p. (1)

1444. PEPPAR, J. L., AND P. R. PICKARD. 1978. Angling survey of the Cains river, Miramichi river systems, New Brunswick, 1976. 20 p. (8)

1445. CADDY, J. F. 1977. Approaches to a simplified yield-per-recruit model for crustacea, with particular reference to the American lobster (*Homarus americanus*). 14 p. (6)

1446. BREEN, P. A., B. E. ADKINS, AND D. C. MILLER. 1978. Recovery rate in three exploited sea urchin populations from 1972 to 1977. 27 p. (1)

Rapports manuscrits

La série comprend des articles d'information et des rapports préliminaires non publiés, qui ne sont pas destinés à la distribution générale. Ils ne doivent pas être donnés en références sans l'assentiment du service dépositaire et sans une indication claire de leur forme manuscrite.

1447. DAVIS, J. C., I. G. SHAND, G. CHRISTIE, AND G. KOSAKOSKI. 1978. Biological and oceanographic observations in the Neroutsos Inlet area with emphasis on the effect of sulfite pulp mill waster on Pacific salmon, 1973–1977. 88 p. (1)

1448. MAJEWSKI, H. S., R. W. DANELL, J. BARICA, J. F. KLAVERKAMP, AND A. LUTZ. 1978. Short-term exposure to rainbow trout (*Salmo gairdneri*) to an algicidal level of copper sulphate in two hard-water ponds. 10 p. (4)

1449. BOND, W. A., R. W. MOSHENKO, AND G. LOW. 1978. An investigation of walleye, *Stizostedion vitreum vitreum*, (Mitchill) from the sport fishery of the Hay River, Northwest Territories, 1975. 19 p. (4)

1450. SHEPHERD, B. G. 1978. Biological reconnaissance of Mathers and Pallant creeks to December, 1977. 89 p. (12)

1451. WRIGHT, D. G. 1978. Observations on the environmental monitoring and surveillance of the Alyeska or Trans-Alaska pipeline. 26 p. (4)

1452. BANFORD, C. 1978. Heath ray incubation and rearing of steelhead trout *Salmo gairdneri* at Fulton river. 19 p. (12)

Data Reports

These reports provide a medium for filing and archiving data compilations where little or no analysis is included. Such compilations commonly will have been prepared in support of other journal publications or reports. The subject matter of Data Reports reflects the broad interests and policies of the Fisheries and Marine Service, namely, fisheries management, technology and development, ocean sciences and aquatic environments relevant to Canada.

Numbers 1–25 in this series (except No. 24 issued by Newfoundland Region, St. John's, Nfld.) were issued as Fisheries and Marine Service Data Records by the Pacific Biological Station, Nanaimo, B.C. The series name was changed with report number 26.

Rapports statistiques

Ces rapports servent de base à la compilation des données de classement et d'archives pour lesquelles il y a peu ou point d'analyse. Cette compilation aura d'ordinaire été preparée pour appuyer d'autres publications ou rapports. Les sujets des Rapports statistiques reflètent la vaste gamme des intérêts et politiques du Service des pêches et de la mer, notamment gestion des pêches, techniques et développement, sciences océaniques et environnements aquatiques, au Canada.

Les numéros de 1 à 25 de cette serie (sauf le nº 24, publié par région de Terre-Neuve, St-Jean, T.-N.) ont été publies à titre de Records statistiques, Service des pêches et de la mer, par la Station biologique de Pacifique, Nanaïmo, (C.-B.). Le nom de la série a été modifie à partir du numéro 26.

63. HARBO, R. M., AND I. K. BIRTWELL. 1978. Mercury content data for crustaceans and fishes from Howe Sound, British Columbia, May 1977 to April 1978. 20 p. (12)
64. MACPHAIL, D. K., AND J. C. CALDER. 1978. Water quality survey, Sherbrooke Lake, Nova Scotia. 40 p. (8)
65. BALLANTYNE, A. 1978. Graphs of sea levels at four British Columbia stations: Alert Bay, Tofino, Point Atkinson, Prince Rupert. 19 p. (1)
66. BARNER, L. W., AND F. H. C. TAYLOR. 1978. Midwater trawl tows and catches made on G. B. REED cruises GBR 74-1, -2, GBR 74-4, -5 off the southeast coast of Vancouver Island. 47 p. (1)
67. BALLANTYNE, A. 1978. Surface and bottom temperatures obtained during the 1977 XBT monitoring program. 53 p. (1)
68. WICKETT, W. P., J. A. THOMSON, AND A. BALLANTYNE. 1978. Transport computations for the north Pacific ocean, 1977. 127 p. (1)
69. SCARSBROOK, J. R., P. L. MILLER, J. M. HUME, AND J. MCDONALD. 1978. Purse seine catches of sockeye salmon (*Oncorhynchus nerka*) and other species of fish at Babine Lake, British Columbia, 1977. 41 p. (1)
70. MCLEAN, W. E. 1979. Quinsam salmon hatchery background water quality data, 1972–1978. (1)
71. WEIR, K. R., R. J. BEAMISH, M. S. SMITH, AND J. R. SCARSBROOK. 1978. Hake and pollock study, Strait of Georgia bottom trawl cruise, G. B. REED February 25–March 13, 1975. 153 p. (1)
72. MARSHALL, D. E., R. F. BROWN, V. D. CHAHLEY, AND D. G. DEMONTIER. 1979. Preliminary catalogue of salmon streams and spawning escapements of statistical area 2E (Queen Charlotte Islands). (2)
73. SHEARER, J. A., AND D. R. DECLERCQ. 1978. Light extinction measurements in the Experimental Lakes Area — 1977 data. 49 p. (4)
74. DECLERCQ, D. R., AND J. A. SHEARER. 1978. Phytoplankton primary production, chlorophyll and suspended carbon in the Experimental Lakes Area — 1977 data. 62 p. (4)
75. HAEGELE, C. W., AND R. D. HUMPHREYS. 1978. Data record for 1977 herring spawn studies. 135 p. (1)
76. PERCY, J. A., P. VERMETTE, P. BOUCHARD, AND J. FIFE. 1978. Osmoregulation and water permeability of arctic marine isopods *Mesidotea entomon, M. sibirica* and *M. Sabini*. 163 p. (5)
77. BEAMISH, R. J., C. WOOD, AND C. HOULE. 1978. A summary of sablefish tagging studies conducted during 1977. 103 p. (1)
78. HARLING, W. R., R. M. WALLIS, M. R. FRETWELL, AND F. W. MOTTL. 1978. Arctic Harvester Groundfish Cruise no. 78-3 (February 27–March 10, 1978). 35 p. (1)

Industry Reports

These reports contain the results of research and development that are useful to industry for either immediate or future application. Industry Reports are directed primarily towards individuals in the primary and secondary sectors of the fishing and marine industries. No restriction is placed on subject matter and the series reflects the broad interests and policies of the Fisheries and Marine Service, namely fisheries management, technology and development, ocean sciences and aquatic environments relevant to Canada.

Industry Reports may be cited as full publications. The correct citation appears above the abstract of each report. Each report will be abstracted in *Aquatic Sciences and Fisheries Abstracts* and will be indexed annually in the Service's index to scientific and technical publications.

Numbers 1–91 in this series were issued as Project Reports of the Industrial Development Branch, Technical Reports of the Industrial Development Branch, and Technical Reports

Rapports à l'industrie

Ces rapports contiennent les résultats des recherches et des progrès pouvant être utiles à l'industrie pour des applications soit immédiates, soit futures. Ils sont principalement préparés à l'intention des membres des secteurs primaire et secondaire de l'industrie des pêches et de la mer. Il n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques du Service des pêches et de la mer, notamment: la gestion des pêches, les techniques et la mise en valeur, les sciences océaniques et l'environnement aquatique au Canada.

Les Rapports à l'industrie peuvent être considérés comme des publications complètes. La titre exact paraît au haut du résumé de chaque rapport devant être publié dans la revue *Aquatic Sciences and Fisheries Abstracts* et noté dans l'index annuel des publications scientifiques et techniques du Service.

Les numéros 1 à 91 de cette série ont été publiés à titre de rapports sur les travaux de la Direction du développement

of the Fisherman's Service Branch. The series name was changed with report number 92.

104. BAILEY, R. F. J. 1978. Whelks on snow crab fishing grounds in the southwestern Gulf of St. Lawrence. 9 p. (6)
105. PROVERBS, T. B. 1978. Foreign investment in the British Columbia fish processing industry. 75 p. (12)

Interpretive Articles Series

This new series commenced in 1966. The articles interpret work already published in primary journals. They appear at irregular intervals in various technical publications. Frequently an article essentially duplicates, condenses, or is based on an article in another series.

400. WALDICHUK, M. 1977. Canadian activities in the Pacific Region related to ocean science and marine pollution research, p. 544–561. *In* C. L. Dawson [ed.] The North Pacific Project. Proceedings of a Review Conference, University of Washington, July 27–29, 1977. Inst. Mar. Res., Univ. Wash. Seattle, Wash., IMS Publ. Ser. IMS-UW-77-1: 632 p. (3)
401. WALDICHUK, M. 1977. Estuarine development and ecology, p. 158–194. *In* Prairies to Pacific — The transportation challenge. Proc. 4th Annu. Western Regional Conf. Engineering Inst. Can., March 24–26, 1977, Victoria, B. C. 230 p. (3)
402. WALDICHUK, M. 1978. Book review of: "R. Johnson [ed.] Marine pollution. Academic Press, London, 729 p." Earth Sci. Rev. 14: 87–90. (3)
403. PENROSE, W. R. 1978. Specific biological methods for petroleum baseline and pollution monitoring. Mar. Pollut. Bull. 9: 231–234 (9)

Translation Series

The following translations have been prepared by or for the Fisheries and Marine Service, for use in its investigations. Copies are available from Translation Services, Canada Institute for Scientific and Technical Information, National Research Council of Canada, Ottawa, Ontario K1A 0S2, at a cost of 22¢ per page and a minimum charge of $2.20. Information on translations can be obtained from the Scientific Information and Publications Branch.

Lists of articles in the Translation Series from 4176 to 4450, with index to authors, are available free from the Department of Fisheries and Oceans, Scientific Information and Publications Branch, 240 Sparks St., Ottawa, Ont. K1A 0E6.

HILLEMAN, G. 1978. Experiments with fish oils and vitamin D in mink feed. Dan. Pelsdyravl. p. 245–246. (Transl. from Danish by Fish. Mar. Serv. Transl. Ser. No. 4451, 1978).

GAMS, H. 1927. The history of the Lunz Lakes, bogs and forests. Preliminary communications. Int. Rev. Gesamten Hydrobiol. Hydrogr. 18: 305–387. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 4452, 1978).

TAKEUCHI, T., T. WATANABE, AND C. OGINO. 1978. Supplementary effect of lipids in a high protein diet of rainbow trout. Nippon Suisan Gakkaishi 44: 677–681. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 4453, 1978).

Articles explicatifs

Cette série a débuté en 1966. Les articles donnent une explication de travaux déjà publicés dans des revues prioritaires. Ils paraissent à intervalles irréguliers, dans diverses publications techniques. Souvent, un article se basera sur un écrit d'une autre série, le condensera ou le reproduira presque tel quel.

Traductions

Les traductions dont il est question ci-après ont été préparées par ou pour le Service des pêches et des sciences de la mer, à des fins de recherche. On peut en obtenir des exemplaires, à 22¢ la page et à un prix minimum de $2.20 aux Services de traduction, Institut canadien de l'information scientifique et technologique, Conseil national de recherches du Canada, Ottawa (Ontario), K1A 0S2. La Direction de l'information et des publications scientifiques peut fournir des renseignements sur ces traductions.

On peut se procurer gratuitement la liste des articles de la série Traductions, de 3926 à 4175, avec index par auteurs, à l'adresse suivante : Ministère des Pêches et Océans, Direction de l'information et des publications scientifiques, 240, rue Sparks, Ottawa, Ont. K1A 0E6.

SCHOENHEIMER, R. 1932. The specificity of cholesterol absorption and its biological significance. Klin. Wochenschr 11: 1793–1796. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 4454, 1978).

ANONYMOUS. (Undated). The hatching and release of salmons in the Iwate Prefecture. Association for the Propagation of Salmon in the Iwate Prefecture. (Transl. from Japanese by Fish. Mar. Serv. Transl. Ser. No. 4455, 1978).

BRABANT, J.-C. 1974. Devisme's selective shrimp trawl. Study of a modified selective device. Sci. Pêche. Bull. Inst. Pêches Marit. 236: 1–14. (Transl. from French by Fish. Mar. Serv. Transl. Ser. No. 4456, 1978).

PRZYBOROWSKI, L., AND A. SMAJKIEWICZ. 1978. Com-

plexometric determination of cod-liver oil in ointments. Farm. Pol. 34: 87–89. (Transl. from Polish by Fish. Mar. Serv. Transl. Ser. No. 4457, 1978).

GANOWIAK, Z., R. GAJEWSKA, M. NABRZYSKI, B. WITUSZYNSKA, AND E. LIPKA. 1977. Nutritive value and preliminary hygienic evaluation of krill (*Euphausia superba*) and some Antarctic fish. Ann. Acad. Med. Gedanenis 7: 207–216. (Transl. from Polish by Fish. Mar. Serv. Transl. Ser. No. 4458).

TANIKAWA, E., ET AL. 1956. Studies on the complete utilization of squid (*Ommastrephes sloani pacificus*). XIV. Relation between the water content of squid meat and putrefaction during the drying of squid. Hokkaido Daigaku Suisan Gakubu Kenkyu Iho 7: 172–184 (Transl. from Japanese by Fish. Mar. Serv. Transl. Ser. No. 4459, 1979).

TANIKAWA, E., ET AL. 1956. Studies of the complete utilization of squid. Hokkaido Daigaku Suisan Gakubu Kenkyu Iho 7: 165–171. (Transl. from Japanese by Fish. Mar. Serv. Transl. Ser. No. 4460, 1979).

IKEHARA, K., AND M. NAGAHARA. 1978. Fundamental studies for establishing rockfish culture techniques. VI. On the favourable contents of protein and lipid in compound food for young rockfish, *Sebastes thompsoni* (Jordan et Hubbs). Nihonkai Ku Suisan Kenkyusho Kenkyu (29): 103–110. (Transl. from Japanese by Fish. Mar. Serv. Transl. Ser. No. 4461, 1979).

TAKEUCHI, T., ET AL. 1978. Use of hydrogenated fish oil and beef tallow as a dietary energy source for carp and rainbow trout. Nippon Suisan Gakkaishi 44: 875–881. (Transl. from Japanese by Fish. Mar. Serv. Transl. Ser. No. 4462. 1979).

BERGER, M. 1977. The Silox fish rearing system: a new technique for the intensive production of food fishes. Fischer Teichwirt (9): 4 p. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 4463, 1979).

DITTMAR, K. E. J., ET AL. Gas chromatographic analysis of trans isomeric fatty acids on packed columns (Silar 10C, Silar 9CP, SP 2340, OV 275, and on SP 2340 coated glass capillaries. Fette, Seifen, Anstrichm. 80: 297–303. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 4464, 1979).

List of Establishments

(1) Department of Fisheries and Oceans
Pacific Biological Station
P.O. Drawer 100
Nanaimo, British Columbia V9R 5K6

(2) Department of Fisheries and Oceans
Vancouver Laboratory
6640 N.W. Marine Drive
Vancouver, British Columbia V6T 1X2

(3) Department of Fisheries and Oceans
Pacific Environment Institute
4160 Marine Drive
West Vancouver, British Columbia V7V 1N6

(4) Department of Fisheries and Oceans
Freshwater Institute
501 University Crescent
Winnipeg, Manitoba R3T 2N6

(5) Department of Fisheries and Oceans
Arctic Biological Station
P.O. Box 400
Ste. Anne de Bellevue, Quebec H9X 3L6

(6) Department of Fisheries and Oceans
Biological Station
St. Andrews, New Brunswick E0G 2X0

(7) Department of Fisheries and Oceans
Marine Ecology Laboratory
Bedford Institute
P.O. Box 1006
Dartmouth, Nova Scotia B2Y 4A2

(8) Department of Fisheries and Oceans
Maritimes Regional Library
P.O. Box 550
Halifax, Nova Scotia B3J 2S7

(9) Department of Fisheries and Oceans
Research and Resource Services
Newfoundland Region
3 Water Street
St. John's, Newfoundland A1C 1A1

(10) Department of Fisheries and Oceans
Scientific Information and Publications Branch
240 Sparks Street
Ottawa, Ontario K1A 0E6

(11) Department of Fisheries and Oceans
Canada Centre for Inland Waters
Great Lakes Biolimnology Laboratory
P.O. Box 5050
867 Lakeshore Rd.
Burlington, Ontario L7R 4A6

(12) Department of Fisheries and Oceans
Pacific Region
1090 West Pender Street
Vancouver, British Columbia V6E 2P1

Liste d'établissements

(1) Ministère des Pêches et des Océans
Station biologique du Pacifique
C.P. 100
Nanaïmo (Colombie-Britannique) V9R 5K6

(2) Ministère des Pêches et des Océans
Laboratoire de Vancouver
6640 nord-ouest, prom. Marine
Vancouver (Colombie-Britannique) V6T 1X2

(3) Ministère des Pêches et des Océans
Institut de l'environnement du Pacifique
4160, prom. Marine
Vancouver-Ouest (Colombie-Britannique) V7V 1N6

(4) Ministère des Pêches et des Océans
Institut des eaux douces
501, University Crescent
Winnipeg (Manitoba) R3T 2N6

(5) Ministère des Pêches et des Océans
Station biologique de l'Arctique
C.P. 400
Saint-Anne-de-Bellevue (Québec) H9X 3L6

(6) Ministère des Pêches et des Océans
Station biologique
St. Andrews (Nouveau-Brunswick) E0G 2X0

(7) Ministère des Pêches et des Océans
Laboratoire d'écologie marine
Institut de Bedford
C.P. 1006
Dartmouth (Nouvelle-Écosse) B2Y 4A2

(8) Ministère des Pêches et des Océans
Bibliothèque de la région des maritimes
C.P. 550
Halifax (Nouvelle-Écosse) B3J 2S7

(9) Ministère des Pêches et des Océans
Services des recherches et des ressources
Région de Terre Neuve
3, rue Water
St-Jean (Terre-Neuve) A1C 1A1

(10) Ministère des Pêches et des Océans
Direction de l'information et des publications scientifique
240, rue Sparks
Ottawa (Ontario) K1A 0E6

(11) Ministère des Pêches et des Océans
Centre canadien des eaux intérieures
Laboratoire de biolimnologie des Grands Lacs
C.P. 5050
867, ch. Lakeshore
Burlington (Ontario) L7R 4A6

(12) Ministère des Pêches et des Océans
Région du Pacifique
1090, rue West Pender
Vancouver (Colombie-Britannique) V6E 2P1

This publication is available in

Cette publication est disponible en

MICROFORM

from/de

Xerox University Microfilms
300 North Zeeb Road
Ann Arbor, Michigan 48106

Xerox University Microfilms
35 Mobile Drive
Toronto, Ontario,
Canada M4A·1H6

University Microfilms Limited
St. John's Road,
Tyler's Green, Penn,
Buckinghamshire, England

Please write for complete information
Ecrivez pour information plus complète

Journal of the Fisheries Research Board of Canada

Journal de l'office des recherches sur les pêcheries du Canada

JFRBAK36(5)481–604(1979)
ISSN 0015-296X

Volume 36, No. 5, May 1979

Volume 36, nº 5, mai 1979

Timing and magnitude of energy deposition and loss in the body, liver, and gonads of northern pike (*Esox lucius*) **J. S. Diana and W. C. Mackay** 481–487

Detritus and juvenile salmon production in the Nanaimo Estuary: I. Production and feeding rates of juvenile chum salmon (*Oncorhynchus keta*) **M. C. Healey** 488–496

Detritus and juvenile salmon production in the Nanaimo Estuary: II. Meiofauna available as food to juvenile chum salmon (*Oncorhynchus keta*) **J. R. Sibert** 497–503

Detritus and juvenile salmon production in the Nanaimo Estuary: III. Importance of detrital carbon to the estuarine ecosystem **R. J. Naiman and J. R. Sibert** 504–520

Net entanglements of baleen whales in the inshore fishery of Newfoundland **J. S. Perkins and P. C. Beamish** 521–528

Studies on the sediment of St. Margaret's Bay, Nova Scotia **T. G. Hughes** 529–536

Predation of sea scallops, *Placopecten magellanicus*, by the rock crab, *Cancer irroratus*, and the American lobster, *Homarus americanus* **R. W. Elner and G. S. Jamieson** 537–543

Possible adaptive significance of certain enzyme polymorphisms in steelhead trout (*Salmo gairdneri*) **J. M. Redding and C. B. Schreck** 544–551

Population-genetic study of the effects of selective fishing on the growth rate of trout **L. D. Favro, P. K. Kuo, and J. F. McDonald** 552–561

Infectious pancreatic necrosis virus: quantification of carriers in lake populations during a 6-year period **T. Yamamoto and J. Kilistoff** 562–567

Short-term covariability of chlorophyll and temperature in the St. Lawrence Estuary **S. Demers, P. E. Lafleur, L. Legendre, and C. L. Trump** 568–573

Quantification of vitellogenin in Atlantic salmon (*Salmo salar*) plasma by radioimmunoassay **D. R. Idler, S. J. Hwang, and L. W. Crim** 574–578

Sorption of cadmium and its effect on growth and the utilization of inorganic carbon and phosphorus of two freshwater diatoms **H. L. Conway and S. C. Williams** 579–586

Notes

Pathogenesis of infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*) **R. N. Swanson and J. H. Gillespie** 587–591

Rapid decompression stress accelerates fibrinolysis in fingerling salmon **J. J. Woodward, E. Casillas, L. S. Smith, and B. G. D'Aoust** 592–594

Obituary — N. M. Carter **W. E. Ricker** 595–596

Book Reviews/Comptes rendus: A coastal marine ecosystem. Simulation and analysis (J. N. Kremer and S. W. Nixon) **W. L. Silvert** 597–598

Coming next month/À paraître le mois prochain 599

Recent publications/Récentes publications 600–604

©Minister of Supply and Services Canada 1979
Ministre des Approvisionnements et Services Canada 1979